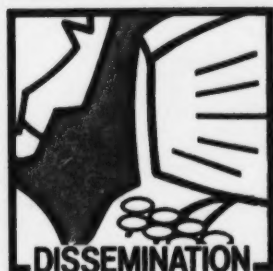


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SPECIAL LISTING

CURRENT CANCER RESEARCH

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RADIATION
CARCINOGENESIS AND
RELATED RADIOBIOLOGY

APRIL 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service • National Institutes of Health •
National Cancer Institute

A SERVICE OF THE
INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB)

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Dear Cancer Researcher:

This **SPECIAL LISTING** of Current Cancer Research Projects is a publication of the INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB) Program of the National Cancer Institute. Each **LISTING** contains descriptions of ongoing projects in one selected cancer research area. Approximately 50 different cancer research areas are covered by a separate **SPECIAL LISTING**.

The present series of **SPECIAL LISTINGS** is a third edition, and begins with number 201. This series will update the second edition which included **SPECIAL LISTINGS** numbered 101 through 147. Depending on the total number and types of cancer research summaries received, the third edition will have at least 47 titles, and possibly a few more. In most cases, each third edition **SPECIAL LISTING** will replace the exact subject area of a second edition **LISTING** (SL 201 replacing SL 101, etc.). Whenever the number and variety of summaries requires a change in this procedure, a notation will be made in the specific **LISTINGS** affected.

The purpose of these **LISTINGS** is to promote and facilitate the exchange of information among cancer scientists. The project descriptions in the **LISTINGS** are provided by cancer scientists from more than 50 different countries. Researchers are urged to prepare accurate summaries which will be rapidly processed for inclusion in the CANCERPROJ data base as well as future **SPECIAL LISTINGS**.

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CURRENT CANCER RESEARCH

I. HUMAN CANCER AND EXPOSURE TO RADIATION

A. ONCOGENESIS AND THERAPEUTIC EXPOSURE TO RADIATION

1. IATROGENIC EXPOSURE TO X-RAYS AND THE INCIDENCE OF LEUKEMIA

1. RELATIONSHIP BETWEEN LEUKEMIA AND EXPOSURE TO DIAGNOSTIC RADIATION

Majle, T., Landau, E. Z., Lundin, J., National Inst. of Hygiene, Dept. of Radiation Protection & Radiobiology, 24 Chocimska St., 00791 Warsaw 36, Poland

The Tri-State Leukemia Study in U.S.A., 1962-1963, showed an excess of leukemias in adult males associated with an increased frequency of x-ray examinations. The purpose of the present study is to validate the results of the Tri-State Leukemia Study and to estimate exposure and dose of x-rays in 1,000 leukemia patients and in 1,000 controls chosen by random sampling from the Polish population. A retrospective survey provides information on the number and kind of x-ray examinations during the past 20 years. Information obtained from patients and controls is confirmed in hospitals, polyclinic records and archives. Exposure and dose of x-rays will be compared in both groups. A pilot study in Warsaw of 225 leukemia cases and 419 controls was completed in 1978. Some of the results show that the radiation load in leukemia patients may be higher than in relevant control groups. The results in children seem to indicate that children with leukemia were subjected to a higher number of x-ray examinations than the controls.

2. RADIATION-INDUCED CHRONIC MYELOGENOUS LEUKEMIA

Shimaoka, K., Sokal, J. E., Roswell Park Memorial Inst., Dept. of Medicine, 666 Elm St., Buffalo, New York, 14263, United States of America

The aim of this study is to identify patients with chronic myelogenous leukemia who have previous radiation exposure (including in utero and early infancy). These patients will be analyzed for manifestations, clinical course and therapeutic response, in comparison to chronic myelogenous leukemia patients without radiation exposure.

3. LOW DOSE RADIATION AND LEUKEMIA

Linos, A., Gray, J. E., Orvis, A. L., Kyle, R. A., O'Fallon, W. M., Mayo Clinic, Dept. of Medical Statistics & Epidemiology, 200 1st St. S.W., Rochester, Minnesota, 55901, United States of America

All diagnosed cases of leukemia in Olmsted County residents between 1955 and 1974 (a total of 138 cases) have been identified. Each has been appropriately matched with two controls. Cases and controls are being compared with regard to their exposures to radiation for therapeutic, diagnostic, and other reasons. The effect of a life-long experience of low-level radiation (less than 300 rads to the bone marrow) on the development of leukemia is being investigated.

4. RADIATION CARCINOGENESIS

Neumeister, K., Staatliches Amt fuer Atomsicherheit und Strahlenschutz, Waldowallee 117, 1157 Berlin, German Democratic Republic

Epidemiological analyses are undertaken of radiation-induced carcinoma and leukemia following diagnostic or therapeutic administration of ionizing radiation, including pre- and

postnatal exposure in children, and of radiation-induced cancer in multiple primary malignant tumors in the German Democratic Republic.

5. REANALYSIS OF THE TRI-STATE LEUKEMIA SURVEY DATA

Ginevan, M. E., U.S. Dept. of Energy, Argonne National Lab., 9700 S. Cass Ave., Argonne, Illinois, 60439, United States of America

The objective is to perform a re-analysis of the adult portion of the tri-state data, accomplishing the following specific tasks: (1) calculate age-standardized relative risks of adult leukemia for non-overlapping x-ray exposure categories and develop absolute risk estimates which can be related to existing absolute risk estimates for low-level low-LET radiation leukemogenesis; (2) investigate possible relationships between radiation exposure, disease, and leukemogenesis in adults and discuss these findings in the context of susceptible subgroups for radiation leukemogenesis; (3) investigate the possible influence of other variables, e.g., temporal aspects of exposure, ethnic, socioeconomic on the relationship between diagnostic x-ray exposure and leukemogenesis; and (4) develop explanatory models and generate testable hypotheses to account for the tri-state data.

6. RE-ANALYSIS OF THE TRISTATE LEUKEMIA SURVEY DATA WITH SPECIAL REFERENCE TO THE LEUKEMOGENIC POTENTIAL OF DIAGNOSTIC X-RAYS

Ginevan, M. E., Curtiss, J. R., Grahn, D., Argonne National Lab., Division of Biological & Medical Research, 9700 S. Cass Ave., Argonne, Illinois, 60439, United States of America

The data under study consist of 1,414 adult leukemia cases and 1,370 controls matched for age and sex. Earlier analyses of the data suggest considerable uncertainty as to the exact nature of this association. Analyses to date suggest that there is a precipitous increase in risk of myelogenous leukemia in males exposed to 40 or more X-ray films within a 10-year period. This apparent association is being investigated to determine its exact nature. Among the hypotheses being examined is that the excess leukemia risk is a result of preleukemic conditions which elevate the probability of being X-rayed (i.e. leukemia causes X-rays) versus the possibility that ill health results in X-rays being taken which cause leukemia. To this end the disease histories of leukemia cases and controls are being compared, as are the temporal aspects of their X-ray exposure histories. A complete re-analysis of the entire data set is also being carried out, which it is hoped will contribute significantly to the understanding of low level, low linear energy transfer, radiation risks.

7. NATIONAL SURVEY OF CHILDHOOD CANCERS

Kinnier Wilson, L. M., Stewart, A. M., Draper, G., Doll, R., Marie Curie Memorial Foundation, Epidemiology Unit, The Chari, Oxted RH8 0TL, England, United Kingdom

This long-standing retrospective survey was originally started in Oxford by Dr. A. Stewart in 1953 to study the aetiology of childhood cancers. It consists of interviews with the parents of children under 16 dying of cancer or leukemia in the United Kingdom and with parents of control children matched for age, sex and district; antenatal information is confirmed by application to clinics and doctors, and the case child's medical history is followed up through general practitioner and hospital notes. Surviving children have similarly been interviewed and followed up since 1962 by means of the regional cancer registries. In the past extensive studies have been made of the relation of obstetric low-level radiation to the increased risk of the child later developing neoplasia. The effect of viral diseases during pregnancy has also been found to increase the risk, but less

convincingly. Present projects include: 1. A study of the effects of the use of ultrasound and of drug therapy during pregnancy, and of the possible relationship of these agents in the aetiology of childhood cancers. 2. A study of congenital defects found in children who later develop cancer or leukemia, and congenital defects in their siblings, together with a similar study of defects in matched control children and their siblings. 3. A study of all cases of Wilms' tumor, surviving and dead, whose diagnosis and initial treatment occurred in 1969-1973 to assess the difference in prognosis between those who were included in the M.R.C. Trial and those outside. This study is being done in collaboration with the C.C.R.G. in Oxford. 4. A study of 95 second primary tumors occurring in children after cure of their initial cancer is being undertaken with special attention to the effect of radiation and genetics.

8. NATIONAL SURVEY OF CHILDHOOD CANCERS

Kinnier Wilson, L. M., Waterhouse, J. A., Stewart, A. M., Draper, G. J., Marie Curie Memorial Foundation, Epidemiology Unit, The Chart, Oxted RH8 0TL, England, United Kingdom

This long-standing retrospective survey was originally started in Oxford by Dr. A. Stewart in 1953 to study the etiology of childhood cancers. It consists of interviews with the parents of children under 16 dying of cancer or leukemia in the United Kingdom and with parents of control children matched for age, sex and district; antenatal information is confirmed by application to clinics and doctors, and the case child's medical history is followed up through general practitioner and hospital notes. Surviving children have similarly been interviewed and followed up since 1962 by means of the regional cancer registries. In the past extensive studies have been made of the relation of obstetric low-level radiation to the increased risk of the child later developing neoplasia. The effect of viral diseases during pregnancy has also been found to increase the risk, but less convincingly. Present projects include: (1) A study of the effects of the use of drugs and ultrasound during pregnancy, and of the possible relationship of these agents in the etiology of childhood cancers; (2) A study of parental occupations and their possible relationship to the etiology of childhood cancers; (3) A study of congenital defects found in children who later develop cancer or leukemia, and congenital defects in their siblings, together with a similar study of defects in matched control children and their siblings; (4) A study of second primary tumors occurring in children after cure of their initial cancer with special attention to the effect of radiation and genetics.

9. EPIDEMIOLOGY OF CHILDHOOD MALIGNANCIES IN JAPAN

Hirayama, T., Ministry of Health & Welfare, Epidemiology Div., Tsukiji 5-1-1, Chuo-ku, Tokyo 104, Tokyo, Japan

Patterns of occurrence of childhood malignancies in Japan are being studied in relation to selected risk factors based on data from nationwide registration. A total of 10,575 children (5,951 males; 4,624 females) under age 15 were registered 1969-1977. Malignancies included 4,607 leukemia, 750 malignant lymphoma, 1,002 neuroblastoma, 485 Wilms' tumor, 230 liver tumor, 892 retinoblastoma. Age-specific incidence rates were calculated for each. When compared with vital statistics figures for all Japan, maternal ages had little or no influence, but significant risk elevation was noted under age three with increase in weight at birth, for leukemia, neuroblastoma, Wilms' tumor, testis tumor, and teratoma. Abdominal radiation exposure during the latter part of pregnancy was noted to enhance leukemia risk when compared with 1,011 controls of similar age. Down's syndrome was frequent in leukemia under age three and many cases of congenital malformation including seven hemihypertrophy, five aniridia, six congenital heart disease, were recorded co-existent with Wilms' tumor. The mean ages for retinoblastoma were five, six and seventeen months for bilateral, unilateral with family history, and unilateral without family history cases respectively. Analysis continues.

10. GENETIC DAMAGE FROM PRECONCEPTION DIAGNOSTIC RADIATION

Natarajan, N., Bross, I. D., Roswell Park Memorial Inst., Dept. of Biostatistics, 666 Elm St., Buffalo, New York, 14263, United States of America

The aim of this study is to investigate the cumulative genetic damage in children of parents exposed to radiation prior

to conception and of mother exposed during pregnancy. The data used come from the tri-state leukemia survey carried out in specified areas of the states of New York, Maryland and Minnesota between 1959 and 1962 with information on radiation exposure, various demographic factors, health histories and potential environmental hazards for both leukemia cases and random sample controls. A preliminary analysis with the help of a mathematical model shows that there is severe genetic damage in about 1% of the children whose mothers had exposure to x-rays during pregnancy. Also, in that group the risk of leukaemia shows a 50-fold increase and the risk of certain other diseases a 5-fold increase. Analysis of data on separate maternal and paternal preconception radiation exposures showed similar high risk of leukemia and indicator diseases in a small group of affected children. The cumulative genetic damage from the combination of radiation exposure to the father and mother prior to conception and to the mother during pregnancy is estimated.

2. IATROGENIC EXPOSURE TO X-RAYS AND THE INCIDENCE OF THYROID CANCER

11. MANAGEMENT OF PATIENTS WITH HISTORY OF IRRADIATION TO THE THYROID GLAND

Paloyan, E., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, 5th Ave. & Roosevelt Rd., Hines, Illinois, 60141, United States of America

This series began in 1965 according to a protocol designed to determine the incidence of occult malignancies, bilaterality of cancers, and the potential long term, beneficial effects of total thyroidectomy (in spite of added risks of permanent hypoparathyroidism and recurrent nerve palsy) in patients with thyroid nodules and a history of exposure to head and neck irradiation for benign diseases.

During the past year, 22 patients were added to this series, which now numbers 167. The incidence of carcinoma is approximately 43%, the incidence of multicentricity 40%, figures which are similar to our previous reports. A significant malignancy would have been overlooked in 6 patients, if total thyroidectomy had not been done. The incidence of permanent hypoparathyroidism and recurrent nerve palsy still stands at approximately 1%.

BIBLIOGRAPHIC REFERENCES: Paloyan, E. and Lawrence, A.M.: Thyroid neoplasms after radiation therapy for adolescent acne vulgaris. Arch. Dermatol. 114: 53-55, Jan. 1978. Paloyan, E., Prinz, Richard A., and Lawrence, Ann M.: Current Trends in Endocrine Surgery. Surgery Annual 12:205-222, 1980.

12. RADIATION-INDUCED THYROID CANCER

Schneider, A. B., Frohman, L., Pinsky, S., University of Chicago, Michael Reese Hospital & Medical Center, Dept. of Medicine, 2929 S. Ellis Ave., Chicago, Illinois, 60616, United States of America

The major objective of this research proposal is to study radiation-induced thyroid cancer by a coordinated program of clinical and basic endocrinological investigations. The clinical studies are designed to determine the current risk and continuing incidence of radiation-induced thyroid cancer and to evaluate methods of diagnosis and medical and surgical approaches to treatment. This will be accomplished by a longitudinal study of patients who have been evaluated at Michael Reese Hospital because of a history of childhood head or neck irradiation. Of 5,200 patients who were so treated, 1,700 have been examined and another 1,400 have been contacted. In this population there is a 32% prevalence of nodules and among those who have had surgery 36% have had thyroid cancer. Follow-up examination of patients who were initially normal will provide an estimate of the continuing incidence of radiation-induced thyroid cancer. Follow-up examination of patients who were initially abnormal will provide an evaluation of the various methods of treatment which have been used. Together these will provide the information necessary to develop clinical guidelines for the care of persons with a history of head or neck irradiation.

Laboratory studies will focus on the role of plasma thyroglobulin in the diagnosis of thyroid tumors. Plasma thyroglobulin is elevated in a majority of patients with thyroid nodules and in 12% of patients who have a history of irradiation but who otherwise appear completely normal. This project will deter-

mine whether an elevated thyroglobulin level indicates an especially high risk for the formation of thyroid nodules.

We will continue our detailed studies of the molecular properties of plasma thyroglobulin using physical-chemical methods in order to improve its diagnostic usefulness and to understand how it enters the circulation. The Wollman transplantable thyroid tumor line 1-IC2 will be used as a model system for the study of plasma thyroglobulin and thyroid cancer.

BIBLIOGRAPHIC REFERENCES: Schneider, A.B., Stachura, M.E., Arnold, J., Arnold, M., and Frohman, L.A.: Incidence, Prevalence and Characteristics of Radiation Induced Thyroid Tumors. *American Journal of Medicine*. 64:243-252, 1978. Schneider, A.B., and Pervos, R.: Radioimmunoassay of Human Thyroglobulin: Effect of Antithyroglobulin Autoantibodies. *Journal of Clinical Endocrinology and Metabolism* 47:126-137, 1978.

13. RADIATION ASSOCIATED THYROID CARCINOMA

DeGroot, L. J., Refetoff, S., University of Chicago, Pritzker School of Medicine, Dept. of Medicine, 5801 S. Ellis Ave., Chicago, Illinois, 60637, United States of America

An attempt is being made to find patients who had radiation to the thyroid area in childhood, study them intensively by clinical and laboratory means, and detect the presence or absence of thyroid carcinoma. Patients who have possible tumors as a result of radiation are treated by thyroidectomy. The natural history of the thyroid tumors and the value of surgery, prophylactic thyroid hormone suppression, and radioactive iodide therapy of the neoplasms, as well as immunological methods for control of metastatic disease, are being studied.

14. RADIATION-ASSOCIATED THYROID CARCINOMA

Shimaoka, K., Wallace, H. J., Roswell Park Memorial Inst., Dept. of Medicine, 666 Elm St., Buffalo, New York, 14263, United States of America

A program has been instituted to screen annually by clinical and laboratory examinations those persons who have received radiation therapy to the head and neck areas for benign conditions. Those found to have goitre are further evaluated and treated by suppressive therapy with thyroid hormone. Those who respond are continued on long-term drug therapy, while those whose goitres increase in size are offered thyroidectomy. The natural history of radiation-associated benign and malignant thyroid diseases is being studied.

15. HUMAN RADIATION CARCINOGENESIS STUDY

Woodard, E. D., Hempelmann, L. H., Nitzkin, J. L., University of Rochester, School of Medicine & Dentistry, Dept. of Radiology, 601 Elmwood Ave., Rochester, New York, 14627, United States of America

For the past 25 years the health status of the following three populations of persons given radiation treatment for benign conditions many years before has been followed: (1) 2,872 persons given x-ray in infancy for thymic enlargement and their 5,055 untreated siblings; (2) 606 women given x-ray treatment for acute post-partum mastitis controlled by 554 untreated sisters and by 539 women with a history of acute post-partum mastitis not treated with x-ray treatment and 206 of their untreated siblings; and (3) 971 persons given x-ray treatment to the head during childhood for lymphoid hyperplasia of the nasopharynx, 417 persons treated with intranasal radium capsules for the same condition and the 2,751 non-irradiated siblings of the irradiated subjects. In the thymus-irradiated series (1), an increased incidence of neoplasms and of illnesses with autoimmune features was observed over that seen in the non-irradiated siblings and in the general population. Only thyroid neoplasms occurred in sufficient numbers to permit analysis of incidence as a function of dose and other factors. Radiation-induced thyroid cancer shows a linear dose-response. Jews and females have an increased risk of developing thyroid cancer. In the breast-irradiated women (2), the incidence of breast cancer is increased over that in the controls beginning 15 years post exposure and shows a dose-response with an apparent peak at 400 r (air). A histopathological comparison review of breast cancers experienced by the irradiated women and by controls indicates no differences. A program of periodic mammographic-thermographic screening has been implemented in this series. In 1961, the head-irradiated population (3) was shown to have an

increased incidence of radiation-induced neoplasms. They are being resurveyed at the present time.

16. HUMAN RADIATION CARCINOGENESIS STUDY

Woodard, E. D., Hempelmann, L. H., Shore, R., Odoroff, C., Cooper, R., Goh, K., Nitzkin, J. L., University of Rochester, School of Medicine & Dentistry, Dept. of Radiology, 601 Elmwood Ave., Rochester, New York, 14627, United States of America

This application proposes continuation of long-term prospective studies of three well-defined populations irradiated for benign conditions: (1) persons whose thymus glands were irradiated in infancy; (2) persons given x-ray and/or radium treatments for hypertrophy of adenoidal tissue during childhood; and (3) women whose breasts were treated with x-rays for acute post-partum mastitis. The objective of the program is to determine the risk of radiation-induced disease (neoplasia, autoimmune and psychiatric illness) with particular attention to the dose-response relationship for neoplastic disease. A second objective is to detect (by periodic mammographic-thermographic screening) and treat early cases of breast cancer in a high-risk population (mastitis-irradiated series). Additionally, findings in the screening program will be evaluated for predictive significance.

The health of the irradiated subjects and their non-irradiated sibling controls is followed by mailed health questionnaire. Significant illnesses and operations are verified with the attending physician and/or hospital. Sections of neoplasms of selected sites (breast, thyroid, and salivary glands) are requested for review. The breast-irradiated women and eligible controls (NCI guidelines) will be offered annual mammographic-thermographic screening. Laboratory studies of B and T lymphocytes will be conducted on selected individuals in the thymus series. Additional dosimetry calculations will be performed for selected tissue sites in the head-irradiated series (thyroid, pituitary, and salivary glands) and thymus-irradiated series (breast tissue).

17. NEOPLASMS IN IRRADIATED HUMAN POPULATIONS

Hempelmann, L. H., Woodard, E. D., Shore, R. E., University of Rochester, School of Medicine & Dentistry, Dept. of Radiology, 601 Elmwood Ave., Rochester, New York, 14627, United States of America

This study focuses on three well-defined irradiated populations: the first, persons whose thymus glands were irradiated in infancy; the second, women whose breasts were treated with x-rays for acute postpartum mastitis; and the third, persons given x-ray treatments to the head during childhood. Longitudinal studies of reported morbidity and mortality in these populations and their controls have been ongoing for over 20 years. The effects of irradiation earlier in life on subsequent health remain evident. This application proposes continuation of the longitudinal studies, as well as a program of clinical screening by mammography and thermography for the breast-irradiated series.

The objectives of the study are to determine the quantitative risk and the qualitative nature of radiation-induced neoplastic disease and of diseases with autoimmune features. A second objective is to effect by screening the early detection and treatment of breast cancer in a high-risk population.

A histological review will be conducted on all breast cancers that have occurred.

BIBLIOGRAPHIC REFERENCES: Shore, R.E., Hempelmann, L.H., Kowaluk, E., Mansur, P.S., Pasternak, B.S., Albert, R.E., and Huaghie, G.E., Breast Neoplasms in Women Treated with X-rays for Acute Postpartum Mastitis, *JNCI*, 59: 813-822, 1977.

18. IRRADIATION-INDUCED HEAD AND NECK TUMORS

van Daal, W. A., Hermans, J., Goslings, B. M., van Vloten, W. A., Sepmeijer, C. F., Vink, M., Ruiter, D., Pauwels, E. K., Academisch Ziekenhuis Leyden, Dept. of Radiotherapy, Rijnburgerweg 10, 2333 AA Leyden, Netherlands

OBJECTIVES: 1. Tracing and treatment of patients at risk for malignant tumors as a late effect of irradiation for benign diseases. 2. Calculation of dose-effect relationship for radiation-induced thyroid nodules. 3. Comparison of findings of palpation, scintigraphy and histologic examination for the resected thyroid glands. 4. Collection of data on the incidence of radiation-

induced tumors of the skin, mouth, nasopharynx, salivary glands, parathyroids and other organs in the head and neck region.

APPROACH: The addresses and/or information on causes of death of 2480 patients, irradiated after 1932 for tuberculous lymph adenitis, hyperthyroidism, papillomas of the larynx, etc., are obtained from the population register offices. The technique of irradiation, dose and number of fractions received by all these patients are known from the original radiation records. Patients are requested to appear for examination of the head and neck organs. The thyroid is examined by palpation and technetium scintigraphy. If abnormalities are detected, further examination and treatment are advised.

PROGRESS: As of December, 1980, 25 percent of the cohort (605 patients) has been examined. These patients are divided in four categories: 1) 257 patients who showed up for clinical examination. 2) 179 patients who are deceased, with 85 percent known causes of death. 3) 87 patients who refused clinical examination. 84 percent of them supplied us with useful information concerning induced diseases. 4) 82 patients who could not be traced.

Every third clinically examined patient showed nodular thyroid disease. However, the number of thyroid carcinomas seems to be far less than expected.

3. IATROGENIC EXPOSURE TO X-RAYS AND THE INCIDENCE OF BREAST CANCER

19. FOLLOW-UP OF BREAST CANCER CASES REPORTED BY NOVA SCOTIA SUBJECTS IN THE FLUOROSCOPY STUDY

Howe, G. R., Burch, J. D., Miller, A. B., National Cancer Inst. of Canada, Epidemiology Unit, c/o University of Toronto, Toronto, Ontario, M5S 1A8, Canada

OBJECTIVE: The study is designed to determine whether any particular risk factors show synergism with respect to radiation in the induction of breast cancer.

APPROACH: This is a case-control study, the cases being those females identified as having developed breast cancer following institutionalization for tuberculosis in Nova Scotia between 1930 and 1952. Controls are being selected from the corresponding group who have not developed breast cancer. A substantial proportion of subjects have received considerable radiation exposure from fluoroscopy used in conjunction with artificial pneumothorax. All subjects are being personally interviewed in their own homes by a single interviewer to ascertain known risk factors for breast cancer other than radiation. The information so obtained will be combined with radiation exposure data obtained in an ongoing cohort project in order to evaluate any synergistic effect which may exist between radiation and other risk factors.

PROGRESS: The majority of respondents have been traced and a number of interviews completed.

20. RADIATION AS A CAUSE OF BREAST CANCER WITH PARTICULAR REFERENCE TO ACNE TREATED BY RADIATION

Simon, N., Silverstone, S. M., Ginsberg, B., City University of New York, Mount Sinai School of Medicine, Dept. of Radiation Research, 5th Ave. at E. 100th St., New York, New York, 10029, United States of America

Women who had previous radiotherapy for acne should be added to the radiation exposures previously known to cause breast cancer - young women fluoroscoped for tuberculosis, survivors of atomic bomb explosions and post-partum irradiated mastitis. 43 patients with cancer of the breast who have had previous irradiation for acne have been identified in our department. The number of women who have had radiation for acne is now being investigated, to attempt to determine the incidence of cancer of the breast in such women. This survey of a population of women has been started as a feasibility study of 1,000 women in New York. The survey will concentrate on radiation histories of patients with cancer of the breast.

21. RISKS AND BENEFITS OF BREAST CANCER SCREENING PROGRAMS

Bailar, J. C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst.,

Editorial Office, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

OBJECTIVE: Reassessment of epidemiological evidence bearing on the benefits of periodic screening of asymptomatic women, and on various risks and costs. Primary attention is given to hazards of radiation carcinogenesis in breasts exposed to x-radiation during mammography.

22. EPIDEMIOLOGY OF BENIGN BREAST DISEASE

Modan, B., Chaim Sheba Medical Center, Bitan 27, Tel Hashomer, Ramat Gan, Israel

To try to determine the characteristics of patients with different types of non-invasive breast lesions, with particular reference to those factors reputed to be preferentially associated with breast cancer, and to compare the findings with our similarly conducted breast cancer study. The proposal consists of two phases: (a) case control interview study of 600 cases of non-invasive breast disease, in terms of such risk factors as age at first birth; menstrual history; menopausal status; parity; height and weight; familial breast cancer selected drug history; dietary intake and previous radiation exposure; (b) an incidence study of non-invasive breast lesions in a total community. In both phases, particular attention will be paid to the relative frequency of distinct histopathological forms in subpopulations at high and low risk sub-populations for breast cancer (i.e., European vs. Asian and African born). The characterization of the breast lesions will take into account the histological pattern, degree of atypia and lymphoreticulo-endothelial response (LRE).

Identifying a high risk population for breast cancer is the utilization of the results.

23. INTERACTION OF RADIATION AND OTHER RISK FACTORS FOR BREAST CANCER

Howe, G. R., Burch, J. D., Miller, A. B., University of Toronto, Medical Sciences Bldg., Toronto, Ontario, M5S 1A8, Canada

The aim is to examine the interaction of radiation and other risk factors in the induction of breast cancer to determine whether certain groups of women are at high risk of radiogenic breast cancer due to the presence of non-radiation factors. A case-control study is being conducted using 100 cases of female breast cancer identified in a cohort study of patients submitted to Canadian tuberculosis sanatoria between 1930 and 1952, many of whom received considerable doses of radiation at fluoroscopy. In Nova Scotia, where the usual procedure for fluoroscopy was for the patient to face the X-ray tube, the breast received very high radiation doses, and many of the women developed breast cancer. Cases treated in that province have been traced and interviewed or, deceased cases, a proxy interview has been carried out. 200 controls also treated in Nova Scotia have been randomly selected from the cohort and these, too, have been traced and interviewed. The information from the interview questionnaire will be used to ascertain presence of the various risk factors for breast cancer, and these in conjunction with the radiation exposure information from the cohort study will be analyzed to provide data on interaction effects. 60 cases and 120 controls have been traced and interviewed to date. Most of the remaining subjects have also been traced and it is estimated that interviewing will be complete by September 1980.

24. SCREENING FOR NEOPLASMS IN IRRADIATED POPULATIONS

Hempelman, L. H., Woodard, E., Shore, R., Rogoff, R., University of Rochester, School of Medicine & Dentistry, Dept. of Radiology, 601 Elmwood Ave., Rochester, New York, 14627, United States of America

This population of 606 women treated with x-rays for acute post-partum mastitis and their controls (554 sisters; 539 women with post-partum mastitis not treated with x-rays, and their 206 sisters) have been followed by mail questionnaire since 1955. The irradiated women have an increased risk of developing breast cancer. This application proposes continuation of the mail health survey, the review of histopathology of the breast cancers experienced by the treated women and the controls, and periodic mammographic-thermographic screening of the treated women and eligible controls (NCI guidelines).

4. IATROGENIC EXPOSURE TO X-RAYS AND THE INCIDENCE OF OTHER CANCERS

25. LONG-TERM FOLLOW-UP OF PERSONS EXPOSED TO SCALP IRRADIATION IN CHILDHOOD

Modan, B., Ron, E., Chaim Sheba Medical Center, Dept. of Clinical Epidemiology, Bitan 27, Tel Hashomer, Ramat Gan, Israel

Approximately 11,000 individuals irradiated between 1948-1960, for control of tinea capitis, and two matched control groups (almost 16,000 people) are being retrospectively followed through national registries and medical records. The irradiated group has been found to have a significant excess of malignant and benign head and neck tumors, especially of the brain, parotid gland and the thyroid.

26. FOLLOW-UP STUDY OF PATIENTS TREATED BY X-RAY EPILATION FOR TINEA CAPITIS

Albert, R. E., Shore, R. E., Pasternack, B. S., New York University, School of Medicine, Dept. of Environmental Medicine, 550 1st Ave., New York, New York, 10016, United States of America

Children treated for tinea capitis between 1940 and 1959 at a major New York City dermatology clinic have been followed to examine the tumorigenic effects of X-radiation to the head (300 - 400 R). Some 2,215 irradiated patients and 1,395 patients given medications for tinea capitis have been traced. The groups are very well matched on sex, race, age and socioeconomic status. A third survey of these groups (average interval since tinea treatment - 20 years) is in progress and is showing a continued excess of basal cell carcinomas, and brain, thyroid and parotid tumors in the irradiated group.

27. DOSE EFFECT RELATIONSHIP OF LOW DOSAGE X-RAY THERAPY TO THE HEAD AND NECK AREA

Dean, G., Adelstein, A. M., Medico Social Research Board, 73 Lower Baggot St., Dublin 2, Ireland

Modan et al. showed that there was an increased mortality from certain cancers among those who had been treated as children with X-ray therapy for ringworm of the scalp (Lancet, i, 277-279, 1974). It is proposed to ascertain the mortality of those who were given superficial X-ray therapy to the head and neck area between 1928 and 1960 for acne and related conditions. Hospitals at Liverpool, Glasgow, Birmingham, Manchester, Leeds and London are collaborating in the study. The main conditions treated were acne, sycosis barbae, other skin conditions and eye conditions. It is expected that information will be obtained for about 10,000 patients treated. Follow-up will be achieved using the National Health Service Register in Southport to find deaths since the start of the National Health Service in 1948 and through the Area Health Authorities and the family doctors, to find those who are still alive in case further study is required at a later date. Both the National Cancer Register and regional cancer registers will also be checked. The study program will be completed in 1981/82.

28. CASE-CONTROL STUDY OF MALIGNANT GLIOMA

Hochberg, F., Cole, P., Massachusetts General Hospital, 32 Fruit St., Boston, Massachusetts, 02114, United States of America

This is a case-control study of 230 newly diagnosed cases having a malignant (grades II-IV) glioma. The controls (230) are the 'best friends' of the cases of the same sex and similar age. The controls are further specified by an algorithm. Information is gathered primarily by a mail questionnaire followed by a telephone contact. Interest is in exposure to radiation, childhood diseases and industrial compounds. To date 153 cases and 90 controls have been evaluated.

29. FOLLOW-UP FLUOROSCOPICALLY EXAMINED TUBERCULOSIS PATIENT

Monson, R. R., Harvard University, Cambridge Campus, School of Public Health, Dept. of Epidemiology, Cambridge, Massachusetts, 02138, United States of America

Recontact former Massachusetts tuberculosis sanatoriums and obtain necessary permission and clearances to conduct the study. Select the population to study: both women and men who were discharged alive and who received various treatments for pulmonary tuberculosis. Design forms to abstract medical information and location information from medical records. Abstract medical and location information on all subjected selected

for study. Locate the population selected for study. Design a patient questionnaire to obtain information on current and past disease status, especially cancer history. Mail the questionnaire to all patients located alive. Validate all reported cancer diagnosis. Computerize the medical record data, questionnaire response data, and death certificate data. Determine radiation organ doses as accurately as possible.

30. A STUDY OF CANCER FOLLOWING MULTIPLE FLUOROSCOPIES

Miller, A. B., Howe, G. R., University of Toronto, Medical Sciences Bldg., Toronto, Ontario, M5S 1A8, Canada

This cohort study is designed to evaluate the carcinogenic risk from ionizing radiation in the dose range 0 to 100 rad and for the major cancer sites to establish if there is a possible dose-response relationship. The study population consists of patients first admitted to Canadian tuberculosis sanatoria between the years 1930 and 1952. A substantial proportion (40 to 50%) were fluoroscoped regularly in conjunction with artificial pneumothorax treatment and consequently accumulated a substantial radiation dose consisting of many exposures over a period of time. Information on the admission and treatment records of these patients has been extracted from the original sanatoria files. The data, consisting of approximately 130,000 records, have been keypunched. Two computerized record linkage steps using the identifying information present on the records are currently being undertaken. The first is to internally link the records so that admissions referring to the same individual will be brought together to form a composite record. The second step is to link these composite records to the mortality data base maintained by Statistics Canada for the years 1950 through 1974, in order to ascertain the mortality experience of the cohort. A dosimetry study has been completed and published (Health Physics 33(2):259-70, 1978) which determines the dose experienced by various body organs exposed to radiation from fluoroscopies. The dosimetry information will be applied to the individual patient records in order to estimate the radiation dose received by each patient. The analysis will consist of estimation of relative risks with respect to mortality for different cancer sites as a function of dose calculated relative to those members of the study population who are not fluoroscoped.

31. MALIGNANT NEOPLASMS IN TUBERCULOSIS PATIENTS

Steinitz, R., Costin, C., Israel Center for Registration of Cancer & Allied Diseases, 18-20 King David St., Jerusalem, Israel

A unique opportunity of having two population-based registries under one roof was used for a preliminary investigation of tuberculosis and lung carcinoma which suggested that tuberculosis patients are at a higher risk of developing lung cancer. Possible causes for the high risk were discussed: scar carcinoma, the effect of large amounts of radiation (diagnostic procedures) and the carcinogenic effect of anti-tuberculosis drugs. If radiation is a cause of higher risk, the bulk of the resulting lung carcinomas will have appeared by now, as diagnostic and therapeutic procedures have changed. On the other hand, anti-tuberculosis drugs, especially isoniazid, which has been shown to have carcinogenic effects in animals, have now been in use for a probably sufficient length of time to show their specific carcinogenic effects, not necessarily limited to lung cancer, in tuberculosis patients. The purpose of the study is to re-examine the risk of tuberculosis patients of developing lung and other cancers and to look for differences in the cancer experience of tuberculosis patients according to changes in diagnostic and treatment policies. This is a record linkage study based on the Israel Cancer Registry and the Israel Tuberculosis Registry, supplemented by information from hospital and regional chest clinic records. The Tuberculosis Registry has approximately 55,000 names, starting in 1948, and also includes patients diagnosed before this date. The information on the tuberculosis patients will be extracted from individual records, from the Tuberculosis Registry, hospitals and chest clinics. Information on validation of diagnosis of tuberculosis, date of diagnosis of tuberculosis, localization of the tuberculous process, type and periods of anti-tuberculosis therapy, anatomical site and histology of cancer, date of diagnosis of cancer, activity of the tuberculosis at the time of the diagnosis of the tuberculosis and at the date of diagnosis of the cancer and cause of death will be sought.

32. EPIDEMIOLOGY OF TUMORS OF THE PAROTID GLAND

Preston Martin, S., Casagrande, J. T., Henderson, B. E., University of Southern California, School of Medicine, Dept. of Community & Family Medicine, 2025 Zonal Ave., Los Angeles, California, 90033, United States of America

We propose a case-control study to test the hypothesis that previous exposure to diagnostic dental X-rays and to other forms of radiation are causally related to tumors of the parotid gland. We will also evaluate the importance of other potential risk factors for this disease.

Cases include all types of parotid neoplasms (both benign and malignant) identified by the LAC/USC Cancer Surveillance Program over a five year period. A neighbor control of like sex, race and age (birth year within five years) will be selected for each case. Information on dental and medical X-rays, job exposures, family cancers and tobacco and alcohol use will be obtained through telephone interviews using a standard questionnaire. Interview information on dental X-rays will be validated for a subset of cases and controls by a review of dental record.

The relative importance of various exposures at different ages will be evaluated. Associations of tumor occurrence with radiation exposure and with smoking history will be analyzed separately for tumors of different histologic types.

33. PRIMARY SALIVARY GLAND NEOPLASMS IN ROCHESTER, MINNESOTA

Bouquot, J. E., Lovested, S. A., Weiland, L. H., Elveback, L. R., Kurland, L. T., West Virginia University, School of Medicine, Dept. of Oral Pathology, Morgantown, West Virginia, 26506, United States of America

An association between irradiation of the head and neck and subsequent development of benign and malignant salivary gland neoplasms has been noted in the literature. Most of these neoplasms have been benign. This study will provide such data for the Rochester population as well as detailed clinical information on the few neoplasms that are malignant.

34. CANCER MORTALITY FOLLOWING RADIATION TREATMENT OF ANKYLOSING SPONDYLITIS AND METROPATHIA

Smith, P. G., Doll, R., Imperial Cancer Research Fund, Cancer Epidemiology & Clinical Trials Unit, 9 Keble Rd., Oxford OX1 3QG, England, United Kingdom

This is a retrospective cohort study of mortality among 14,000 patients who were given X-ray therapy for ankylosing spondylitis, and 2,000 women who were given a radiation-induced menopause for metropathia hemorrhagica. The patients were treated in the period 1930 to 1955 and it is planned to continue to compare their mortality experience with that of the general population to study the long term effects of exposure to radiation.

35. CARCINOGENESIS DUE TO RADIATION THERAPY

Blitzer, P. H., Hutchison, G. B., Massachusetts General Hospital, Dept. of Radiation Medicine, 32 Fruit St., Boston, Massachusetts, 02114, United States of America

A study has been initiated of approximately 900 patients with cancers at various sites treated with radiation therapy to determine the incidence of induced neoplasms. The control group will consist of patients with the same primary site and stage treated with surgery.

36. FOLLOW-UP OF PATIENTS TREATED FOR MALIGNANT DISEASE

Alderson, M. R., Alberman, E., Peckham, M. J., Inst. of Cancer Research, Division of Epidemiology, Clifton Ave., Sutton SM2 5PX, England, United Kingdom

This study will follow adolescents and young adults treated with increasingly powerful schedules to quantify the mortality from second malignancies and to check whether there is any evidence of a carcinogenic or teratogenic effect following conception. For both these groups of patients the original medical records will be abstracted; this will include detailed data on initial and subsequent treatment. Where patients have been followed until death, the cause of death will be obtained, whilst those known to be alive will be included, particular effort being made to trace patients presently lost to follow-up. Survivors from adolescence will be interviewed when they have reached

child-bearing age, and a marital history will be obtained including data on any conception that occurs. Information about miscarriage, stillbirth, or children born with congenital abnormalities will be sought; liveborn children will be followed to identify the incidence of malignancy.

37. SYSTEMATIC DETERMINATION OF GENOTYPE OF TWINS AT BIRTH

Derom, R. M., Thiery, M., Boelaert, R., Vlietinck, R., Rijksuniversiteit te Gent, School of Medicine, Dept. of Obstetrics, Pintelaan 135, 9000 Ghent, Belgium

Type, frequency and concordance of congenital malformation. Cancer epidemiology in monozygotic and dizygotic twins. Frequency of malignant conditions in twins which have been exposed to radiation in utero.

38. LATE EFFECTS OF IRRADIATION OF ENLARGED THYMUSES IN CHILDREN

Albert, R. E., New York University, School of Medicine, Dept. of Environmental Medicine, 550 1st Ave., New York, New York, 10016, United States of America

This is part of a broader project. A summary of this subproject is not available.

5. IATROGENIC EXPOSURE TO RADIONUCLIDES AND THE INCIDENCE OF CANCER

39. PROSPECTIVE STUDY OF PATIENTS INJECTED WITH THOROTRAST (PORTUGUESE LONG-TERM SIDE EFFECTS OF THOROTRAST STUDY GROUPS)

Horta, J. S., Cayolla da Motta, L., Tavares, M. H., Universidade de Lisboa, School of Medicine, Cidade Universitaria, Lisbon 4, Portugal

Since the late 1950's neoplastic and other lesions, found mostly in the liver and bone marrow, have been reported in Portuguese patients who were injected some years earlier with thorotrast (a colloidal suspension of thorium dioxide, used mostly as an intravascular radiological contrast medium). A prospective survey of about 2,000 individuals injected with this agent between 1930 and 1950, together with pathological, clinical and laboratory studies, was started in 1961 by the Portuguese Side Effects of Thorotrast Study Group. A significantly higher risk of death from malignancies (mostly liver tumors and acute leukaemia) has been shown among thorotrast recipients compared with controls. This study is continuing. It is hoped that follow-up of these Portuguese patients will continue until the near extinction of surviving patients (now numbering about 200) so that more data relevant to the possible harmful effects to man of other alpha-emitters, such as plutonium, may be collected. The risk of lung and bone cancer is of particular interest with a suggestive increase in bone sarcoma already reported in the literature.

40. LONG-TERM FOLLOW-UP OF PATIENTS EXPOSED TO THOROTRAST

Buckton, K. E., Western General Hospital, Crewe Rd., Edinburgh EH4 2XU, Scotland, United Kingdom

The aims are to assess morbidity and mortality from the isotope, and to study cytogenetic and hematological sequelae.

41. PORTUGUESE STUDY GROUP OF SIDE-EFFECTS OF THORIUM DIOXIDE

Cayolla da Motta, L. C., Tavares, M. H., Saragoca, A., Ferreira, M. A., Ferreira, M. J., National School of Public Health of Portugal, Ave. Padre Cruz a Telheiras, 1699 Lisbon Codex, Portugal

Of 2,436 individuals who have received Thorotrast (a colloidal suspension of ThO₂ used as intravascular radiological contrast medium) in Portugal, mostly between 1930 and 1950, 1,244 (51%) have been traced and followed up, as have 924 of 2,086 (44%) of a control group, injected for the same purpose with non-radioactive contrast material. Of the 955 deaths to 31 December 1976 among Thorotrast cases, 16 died as a direct result of the consequences of local granulomata developed from drug spillage around blood vessels (cervical granulomata in 15 cases), 137 from malignant tumors (of which 6 developed on the edge of local granulomata, 87 being primary tumors - 32 with complete histological confirmation - including 18

hemangioendotheliomas, 4 biliary duct carcinomas, 8 carcinomas of the stomach, 5 of the bronchus and lung, 2 of the larynx, 5 primary bone tumours, and 20 other localizations), 23 died from fatal blood dyscrasias (12 of which were leukemias, mostly acute and myeloid in type), and 27 from liver cirrhosis (actually a particular form of liver fibrosis). Of the 656 control deaths only 15 were caused by malignant tumors (including 4 stomach carcinomas, 1 bronchus, 1 larynx, a 1 probably primary liver tumor); there were no deaths from leukemia or other fatal blood diseases among controls and only 6 deaths were attributed to liver cirrhosis. Statistical analysis of data has shown that the number of observed deaths from malignancies - particularly primary liver, bone, bronchus, and larynx tumors, and leukemia - and from liver cirrhosis in the Thorotrast cases is significantly (0.05) higher than among controls and also higher than the expected corresponding figures in the general Portuguese population. Follow-up of the remaining 300 or so patients and 300 controls will be undertaken as soon as funds are available. Results are expected to contribute, by extrapolation, to a better knowledge and understanding of the human effects of continuous low dose radiation.

42. RISK OF SOMATIC RADIATION DAMAGE - EPIDEMIOLOGICAL INVESTIGATIONS ON RADIATION DAMAGE IN PATIENTS TREATED WITH RA-224

Goessner, W., Spiess, H., Wick, R. R., Gesellschaft fuer Strahlen und Umweltforschung mbH Muenchen, Abteilung fuer Pathologie, Ingolstaedter Landstrasse 1, D8042 Neuherberg, Federal Republic of Germany

In about 2,000 persons treated with radium-224 for ankylosing spondylitis (Bechterew's Disease) or bone tuberculosis, the effects of this particular type of radiation are being investigated. In collaboration with orthopedic hospitals the patients are subjected to a follow-up examination. The results of these investigations, in particular the onset of malignant and degenerative disease and causes of death, are evaluated with a view to establishing dose-response. Further knowledge about the risk of radiation cancers in general is thus gained.

43. METABOLISM AND EFFECTS OF INTERNALLY DEPOSITED RADIONUCLIDES AND TRACE ELEMENTS

Stehney, A. F., U.S. Dept. of Energy, Argonne National Lab., 9700 S. Cass Ave., Argonne, Illinois, 60439, United States of America

The objective is to determine the long-term effects of radium, thorium, and other internal emitters in humans. Epidemiological and clinical studies of human beings who have been occupationally or iatrogenically exposed to intake of alpha-emitting radionuclides will be undertaken. Medical and dosimetric data are obtained by study of living persons and autopsy material and from medical records. The principal end-points are mortality by cause, neoplasms, and pathological changes in bone and lung. Whole body counting (in vivo), radiochemistry, and microscopic techniques are employed. Expected results include identification of diseases associated with internally deposited radionuclides and determination of quantitative relationships between dose and effect. These results are needed for assessment of risk from radionuclides in nuclear fuel cycles.

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44. STUDY OF THE PHYSIOLOGICAL FUNCTION AND HISTOLOGICAL CHANGES OF THYROIDS IRRADIATED WITH RADIOACTIVE IODINE

Dobyns, B. M., Case Western Reserve University, School of Medicine, Dept. of Surgery, 2109 Adelbert Rd., Cleveland, Ohio, 44106, United States of America

The objective is to study the effects of radioiodines on the thyroids of man and animals, with special emphasis on the latent effects, whether from fallout or from diagnostic or therapeutic use. The behavior of therapeutic doses of radioiodine will be examined in man when the radioiodine is given, and later the thyroid failure and the ultimate failure of cell replication will be studied. The development of tumors in patients previously so

treated or in the Marshallese who have been developing tumors from radioiodines in fallout will also be studied. The small doses of ¹³¹I which produce tumors in the thyroid will be explored in animals (rats) and the nature of those tumors will be studied, with special attention to the nature of their evolution. An experimental model has been discovered in rats which produces a high incidence of tumors. The inception and sequence of development of the tumors has been explored. Much has been learned of how the tumors evolve. A high incidence of breast tumors has been found incidentally in these irradiated rats. This is to be explored further. New cases of thyroid tumors are found in the course of studies in the exposed Marshallese each year.

45. STUDIES OF RADIATION-INDUCED CANCER

Land, C. E., Boice, J. D., Blot, W. J., Scotto, J., Stone, B. J., Beebe, G. W., Mason, T. J., Mendelsohn, L., Anderson, G. C., Kleinerman, R., Mundt, D. J., Greene, M. H., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Radiation Studies Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

The objective of this project is to examine cancer incidence and mortality among populations exposed to ionizing and non-ionizing radiation, especially low-dose levels. As pressure mounted to identify the health effects of low-level radiation, a Radiation Studies Section was organized and large-scale studies of irradiated populations initiated. Groups studied include 80,000 Japanese A-bomb survivors, over 100,000 cervical cancer patients treated with radium implants, 20,000 former tuberculosis patients who received multiple chest fluoroscopies, 3,500 children exposed to head and neck irradiation, other patients exposed to diagnostic and therapeutic radiation, workers with histories of occupational exposures to radiation, and the populations of SEER reporting areas with different levels of solar UV radiation. In addition, project members have served on committees and task forces of DHEW, EPA, DOE, NIH, and NCI formulating government responses to current problems associated with health effects of ionizing radiation.

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B. ONCOGENESIS AND OCCUPATIONAL EXPOSURE TO RADIATION

1. CANCER INCIDENCE AMONG URANIUM AND OTHER MINE WORKERS

46. ONTARIO MINERS STUDY

Muller, J., Wheeler, W., Buchanan, R., Gentleman, J. F., Smith, M., Kusiak, R., Ontario Occupational Health & Safety Div., Special Studies & Services Branch, 4000 University Ave., Toronto, Ontario, M7A 1T7, Canada

It is known that uranium miners have an increased lung cancer risk. This study will attempt to calculate the relationship between the time course of exposure to short-lived radon daughters and the time course of excess lung cancer risk. Several control populations will be used: Ontario males, Northern Ontario males and Ontario miners who have not worked in an uranium mine. The study will also investigate cancer risks among non-uranium miners. The employment history data and coded x-ray results (silicosis ratings) from the annual medical examinations required of all active miners in Ontario are being recorded in computer files for all uranium miners and for all miners who have reported for a medical examination in 1955 or later and had 5 or more years of mining experience. In the data files, there are about 16,000 men who qualify as uranium miners and about 34,000 men who meet the latter qualifications. The employment histories and measurements of air concentrations of short-lived radon daughters will be used to make an estimate of each uranium miner's annual exposure between 1955 and 1967. From 1968 onwards exposure was estimated by the mining company. When all these data have been assembled, there will

be, for each uranium miner, a record of exposure to short-lived radon daughters for each year that he was employed as an uranium miner. Information concerning deaths in the study population will be obtained by matching the personal identifying information of each miner with information contained in the Canadian Death Registry maintained by Statistics Canada. The data files will be completely assembled in the early part of 1980 and a review will be started then. The study is on-going and the data are to be reviewed regularly.

47. MORTALITY STUDY OF PRESENT AND FORMER URANIUM MINERS

Hewitt, D., Mueller, J., Wheeler, W., University of Toronto, Dept. of Preventive Medicine & Biostatistics, Medical Sciences Bldg., Toronto, Ontario, M5S 1A8, Canada

This sub-study, commissioned by the Royal Commission on the Health and Safety of Workers in Mines (James M. Ham, Commissioner), has the objective of characterizing the mortality experience of Ontario uranium miners, by certified cause of death, in relation to such variables as duration and intensity of exposure to radon daughters, and time elapsed since exposure.

48. EPIDEMIOLOGICAL STUDIES OF URANIUM MINERS AND MILL WORKERS

Archer, V. E., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance Health Evaluation & Field Studies, 4676 Columbia Pkwy., Cincinnati, Ohio, 45226, United States of America

Uranium miners have been found to have an excess of lung cancer and mill workers to have an excess of lymphomas. A much larger group of mill workers is now being studied by a retrospective cohort study to determine the magnitude of the lymphoma excess. Identification of the substance responsible is being attempted. Two groups of uranium miners are being studied, one of about 4,300, and a much larger one of about 15,000. These groups are being used to determine exposure-response curves, and the different factors which influence the shape of those curves; effects of exposure on the sex-ratio of offspring, the effect of age at first exposure on incidence and latent period, and the dose rate effect.

49. URANIUM EPIDEMIOLOGY STUDY

Buechley, R. W., Key, C. R., Samet, J. M., Morgan, M. V., University of New Mexico, School of Medicine, Epidemiology Section, University Hill N.E., Albuquerque, New Mexico, 87131, United States of America

4,071 uranium miners with at least one year's underground experience since 1957 in the Grants, New Mexico uranium belt will be studied to examine health effects of low level radiation exposures for which only extrapolations are now available. Since mining in this district began, precision of radiation measurement and stringency of radiation standards have increased. Of the 4,071 miners in the study, 1,800 started work before 1961, at mean radiation levels near 40 working level months (WLM) per year. Another 1,300 started in 1961-1970, at mean radiation levels declining over the years from 40 to 4 WLM per year. The remainder started in 1971 or later, at mean radiation levels of 2.5 WLM per year and not exceeding the allowable 4 WLM per year. Standard physical examinations, with cigarette smoking and previous mining histories, are available from the beginning. Of the 139 miner deaths reported to date, 84 were from accidents, poisonings or violence. Of the 55 other deaths, 22 were due to lung cancer, all in miners employed before 1961 in Grants or elsewhere and concentrated in miners with over 200 cumulative WLM. 20 of the 22 lung cancer deaths were in cigarette smokers. Proposed work for 1980-1981 includes determining current status of miners through the US Social Security Administration, etc., refining their amounts of radiation exposure, and planning a very long-term cohort study.

50. URANIUM MINES AND MILLS STUDIES

Archer, V., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance Hazard Evaluations & Field Studies, 4676 Columbia Pkwy., Cincinnati, Ohio, 45226, United States of America

During the period 1950-1975, NIOSH collected data on about 20,000 uranium miners and mill workers. Further study

and analysis of this data is expected to improve the exposure-response curve for lung cancer, to provide data on pulmonary disability and hair root changes, to determine if an altered sex ratio of offspring for uranium miners exists, and to determine if there is an excess of leukemia and cancer of the brain, pancreas, or bone in uranium miners.

Using data from Swedish and other mining groups, a dose-response curve has been constructed extending to quite low levels. This curve suggests that alpha radiation at very low dose rates induces 5 to 10 times as much cancer as equal amounts of radiation at higher dose rates. If this phenomenon is correct, then there are definite implications for setting lower standards, especially for radon daughters in uranium mining and plutonium in the nuclear power industry. Because of importance of the implications, verification or rejection of the present composite exposure-response curve is highly desirable. A retrospective mortality study on new cohort of approximately 3000 uranium miners having less than 12 WLM/year exposure and two control groups will be conducted to test this hypothesis.

51. A STUDY OF URANIUM MINER LUNG CANCER

Saccomanno, G., St. Mary's Hospital & Medical Center, Dept. of Pathology, 7th St. & Patterson Rd., Grand Junction, Colorado, 81501, United States of America

OBJECTIVES: (1) Continuation of sputum collection and collection of lungs from deceased miners. (2) "Predictive" analytical study of sputum samples taken from individual miners over several years. (3) Uranium Miners Lung Study.

APPROACH: (1) Early diagnosis of lung cancer by sputum smears. (2) Many publications of our findings. (3) Manual on Pulmonary Cytology.

RESULTS: (1) Satisfactory technique for preparation of sputum smears. (2) We have proven that carcinoma in situ will remain in that stage for three plus years before becoming invasive.

52. BRONCHUS CARCINOMA IN URANIUM MINERS (SCHNEEBERGER LUNGENKREBS)

Hain, E. F., Ulmer, W. T., Kalthoff, J., Allgemeines Krankenhaus, Dept. of Pneumology, Bissendorfer Pferdeweg 52, 2000 Hamburg 90, Federal Republic of Germany

Bronchus carcinoma is being studied in 47 cases found in uranium miners in the Federal Republic of Germany after the Second World War. 45 of these persons were exposed to uranium in Saxony/Bohemia outside the Federal Republic of Germany. All data, especially the radiation measurements, of these miners are kept secret, so that it is impossible to measure the real radiation exposure. This is estimated by the time of exposure to uranium. No control group is used because of this lack of information. Besides time of exposure the following data are collected: age at death, latency period, place and type of uranium mining, therapy, time of survival after discovery of the carcinoma, site and histology of the carcinoma, metastases, clinical data, pneumoconiosis, and smoking history.

53. EFFECT OF URANIUM ON THE TRACHEOBRONCHIAL TREE

Auerbach, O., Saccomanno, G., Archer, V. E., Kushner, M., Garfinkel, L., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Tremont Ave. & S. Centre St., East Orange, New Jersey, 07019, United States of America

It has been established from epidemiologic studies of uranium miners and asbestos workers that the combination of cigarette smoking and heavy exposure to an occupational carcinogen can significantly increase the risk of lung cancer as compared with smokers without such occupational exposures. It has also been found that the remaining tracheobronchial tree in patients with lung cancer had extensive histological changes compatible with exposures to a carcinogenic agent. These changes include extensive basal cell hyperplasia with cells with atypical nuclei, loss of cilia, carcinoma in situ and early primary invasive cancers presumably not related to the main tumor mass.

We have recently completed a study designed to evaluate the changes in uranium miners compared to non-miners. One hundred and five uranium miners who died of lung cancer were matched on age and smoking habits to non-miners. A total of 28,928 slides were examined. Carcinoma in situ was found in 96 percent of slides in miners and 92 percent of non-miners. Degree 2 and 3 atypias in carcinoma in situ were more commonly found

in the miners. A paper reporting results has been published in *CANCER* 42:483-489, 1978. The data indicate that the synergistic effect of cigarette smoking and uranium exposure increases the risk of lung cancer and that other sites of tissue alteration leading to tumor development are frequently present in the lung.

We are continuing to collect and examine the lungs received from Grand Junction, Colorado, and Butte, Montana, - 383 to date.

54. PREDICTION OF LUNG CANCER RATES FROM SPUTUM CYTOLOGY - EPA

Archer, V., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance Hazard Evaluations & Field Studies, 4676 Columbia Pkwy., Cincinnati, Ohio, 45226, United States of America

Over 50,000 sputum samples have been collected from uranium miners since 1956. The collection has been financed by NIOSH, NCI, and ERDA. The sputum data, along with demographic data, have been put on computer tape and submitted to NCI for analysis. Analysis will be done in several ways, but the most important will be selection of individuals who had certain readings (e.g., Class III) on or before a certain date, then ascertaining deaths among the groups, and doing a life table mortality analysis to determine lung cancer incidence at 5, 10, 15 etc. years. This will be done for most classifications, and will give predictive values for each classification. This will probably apply to other groups as well as uranium miners, but actual study might be needed for verification. Progression from one sputum class to the next higher classification has been demonstrated in many individuals, with a mean of about 5 years between steps. Many individuals show progression. Sputum cytology is now used as a screening service for lung cancer among high risk populations. Many improvements in sputum cytology have come from these studies, and the lives of some uranium miners have been saved.

A contract is planned to collect morbidity and mortality data (and sputum samples where available) on about 500 individuals who have not responded to mailed requests for sputum samples. This will fill a gap in the sputum follow-up which is present in the data. A re-reading of many of the old slides will be done to strengthen the associations. The major activity in this project will be through the proposed contract. It is necessary because the follow-up requires contacting many former miners who are widely scattered. Information must be obtained on changes in their cigarette usage, changes in occupation, changes in sputum cytology, and changes in health.

55. SOMATIC CELL CHROMOSOME CHANGES IN HUMANS EXPOSED TO 239-PLUTONIUM AND 222-RADON

Brandom, W. F., University of Denver, School of Arts & Sciences, Dept. of Biological Sciences, 2115 S. University Blvd., Denver, Colorado, 80210, United States of America

The objective of our study was to determine whether a biological response related to estimated physical doses could be observed in uranium miners' or plutonium workers' cells. If a biological dose-response was observed, the somatic cell chromosome test could provide a biological indicator of a toxicological problem and supplement other indications for setting permissible radiation protection standards. The test system used was the prevalence of chromosome aberrations (dicentric, rings, inversions, translocations, and deletions) in peripheral blood lymphocytes of controls and uranium miners and plutonium workers, plotted against estimated physical radiation doses. Biological responses in the relative prevalence of chromosome aberrations are observed in uranium miners' and plutonium workers' cells. The analysis to date indicates that estimated lung burdens in the plutonium workers are more significant in affecting lymphocyte chromosomes than current estimated systemic burdens. In the future, it is expected that somatic cell chromosome aberration frequencies will be used in conjunction with physical dosimetric measurements to screen other populations exposed to low levels of ionizing radiation.

56. PROSPECTIVE STUDY OF OCCUPATIONAL LUNG CANCER

Baganha, M. F., Bernarda, R. A., Figueiredo, F., Rebelo, R. M., Telo Morais, J. C., Reis, M. V., Leal Goncalves, L. F., Chieira, C. M., Carmo, J. C., Almeida, J. R., Antunes, C. A., Universidade de Coimbra, University Hospital of Coimbra, Clinica de Pneumologia, Coimbra, Portugal

This is a prospective study of workers in a mine with elevated levels of radioactivity involving clinical, radiological, spirometrical, cytological (sputum) and immunological examinations every four months. The results in terms of lung cancer will be compared with those of a group of control workers, who will undergo the same examinations. Both cases and controls will be asked in detail about smoking habits.

57. FOLLOW-UP STUDY OF CANCER IN URANIUM MINERS AND RELATED WORKERS

McMichael, A. J., Clark, P. D., South Australian Health Commission, 52 Pirie St., Adelaide, South Australia 5000, Australia

Approximately 2,600 men operated the Radium Hill uranium mine in South Australia, during 1953-1962. The detailed employment histories of these men, plus the systematic radiation monitoring data form the basis of an on-going follow-up study. Searches of cancer and death registrations throughout Australia, plus searches of voter registrations, driver's licence rolls, and overseas arrivals and departures, give an 80% trace rate. Preliminary proportional mortality analysis of a workforce sample suggests an increase in respiratory cancer.

58. RADIATION EXPOSURE AND MORTALITY IN FLUORSAPAR MINERS

Wigle, D. T., Stocker, H., McCullough, R., Hollywood, B., Canadian Dept. of National Health & Welfare, Lab. Centre for Disease Control, Tunneys Pasture, Ottawa, Ontario, K1A 0L2, Canada

The objective of the study is to evaluate the risk of death due to lung cancer as a function of cumulative exposure to radon and radon daughters in the fluorspar mines of St. Lawrence, Newfoundland. The mortality and adaptation exposure experience of over 2,000 men employed between 1933 and 1977 will be reviewed. Fluorspar mining in St. Lawrence (population 2,078, 1956 census) began in 1933 and by the period 1952-60, lung cancer accounted for 21 of the 71 total deaths: only 0.73 lung cancer deaths were expected based on proportional mortality rates for the province (O/E equals 29). Investigation in 1959 revealed the presence in air of radon and radon daughters at levels up to 193 SWL (1 SWL equals 100 pc/l for radon and 1.3×10 to the 5th power MeV total potential alpha-energy per liter for daughters) in non-working, unventilated areas. The average exposure of miners was estimated to be 2.5-10 SWL. Improved ventilation and monitoring of individual exposure were started in 1960. Current studies are directed towards evaluation of control procedures and analysis of the associations between radiation exposure and mortality utilizing individual exposure data and man-years at risk.

59. MORBIDITY AND MORTALITY AMONG PHOSPHATE MINERS

Matanoski, G. M., Seltzer, R. S., Mitchell, T. M., Billings, C., Block, G. M., Johns Hopkins University, School of Hygiene & Public Health, Dept. of Epidemiology, 615 N. Wolfe St., Baltimore, Maryland, 21205, United States of America

The purpose of this study is to determine whether there is an excess all-cause and cancer morbidity and mortality among employees of a central Florida phosphate mining company. If such an excess is found, a further objective is to attempt to determine a job site or job title which may be associated with the excess. A primary hypothesis is that such an excess may exist due to worker radiation exposure from uranium and daughter products found in phosphate rock.

The approach is nonconcurrent prospective, in which all employees since 1950 are identified, and their outcomes determined. Mortality is ascertained by means of Social Security Administration records and death certificates. Morbidity is ascertained by means of questionnaires and medical record searches. Additional information will be sought from the employees regarding such factors as smoking history, diet, and exposures to other potentially carcinogenic agents or jobs.

An additional aspect of the study involves whole-body radiation counting on a small number of current long-term employees, to determine whether there is evidence of a body burden of radioactive materials.

2. CANCER INCIDENCE AMONG NUCLEAR INDUSTRY WORKERS

60. COMPARATIVE STUDY OF CANCER RISKS FROM EXPOSURE TO EFFLUENTS FROM FOSSIL FUELLED AND NUCLEAR POWER STATIONS

Vohra, K. G., Bhabha Atomic Research Centre, Div. of Radiological Protection, Trombay 400085, Bombay, India

The future large scale production of nuclear power would involve radiation exposure of larger groups of occupational workers, individuals and groups in the population, and the population at large. In view of the concern about the risks of nuclear power often expressed by uninformed public, it is necessary that the risks associated with alternative energy sources such as those based on fossil fuels are compared with similar risks associated with nuclear energy production.

The risks of cancer mortality from ionizing radiations have been studied by two important international Committees, viz. the United Nations Scientific Committee on the Effects of Atomic Radiations and the US Advisory Committee on the Biological Effects of Ionizing Radiations (BEIR Committee). Extensive epidemiological data on the incidence of cancer is also available for several industrial cities, compiled by a number of research institutes. However there has been very little effort in collecting the data of both types at one institution and comparing the two in terms of quantitative risk evaluation.

The proposed program is concerned with the risks of cancer induction associated with the above two energy sources. The risks of specific cancers, for example, lung cancer, would be compared, based on extensive environmental measurements data and epidemiological data.

Risk analysis for radiation induced cancers will be based on the extensive data available on human exposure and animal experiments. The risk associated with fossil fuelled power stations will be assessed on the basis of available data on selected chemical carcinogens and the epidemiological data for urban areas affected by continuous operation of fossil fuelled power stations. The project will also include some measurements of chemical carcinogens in selected areas for direct comparison with epidemiological data.

61. MORTALITY SURVEY OF CEGB RADIATION WORKERS AND EX-WORKERS

Harte, G. A., Wheatley, B. M., Bonnell, J. A., Central Electricity Generating Board, Health Physics Research Section, Berkeley GL13 9PB, Gloucester, United Kingdom

The objective of the study is to determine whether exposure to low levels of ionizing radiation affects cause of death - in particular whether cancer mortality is higher in people occupationally exposed. Data is to be collected initially on 5,000-6,000 ex-workers, but eventually the study will be extended to current radiation workers (marking about 12,000 in all) and the survey will then run continuously. The exposed population will be compared with the general population, the working population, and eventually it is hoped, control groups within the electricity supply industry.

62. LIFETIME HEALTH AND MORTALITY EXPERIENCE OF DEPARTMENT OF ENERGY CONTRACTOR EMPLOYEES WITH OCCUPATIONAL RADIATION EXPOSURE

Gilbert, E. S., Marks, S., Breitenstein, B. D., Hanford Environmental Health Foundation Inc., P.O. Box 100, Richland, Washington, 99352, United States of America

The objectives of this study are to determine whether adverse health effects of exposure to radiation or other agents or substances may be demonstrated in the Hanford employee population, and, if demonstrated, to establish the nature of the effects.

This research program is a joint effort of the Hanford Environmental Health Foundation (HEHF) and the Pacific Northwest Laboratory (PNL). Over-all planning, design of the system of data collection, planning of the analysis of data and

publication of reports are conducted jointly. The collection and processing of data on groups in the worker population, the ascertainment of life status through the Social Security Administration (SSA), and the procurement and coding of death certificates are the responsibility of HEHF. In the latter functions, HEHF may utilize the services of support groups at Oak Ridge Associated Universities. PNL carries out the analysis of the data.

Seven major files are maintained, containing personnel data, work history, external and internal radiation exposure, exposure to other agents (to the limited extent available), medical history, including laboratory and X-ray data, and mortality data for the deceased in the employee roster. Information in the files dates from the onset of plant operation in 1944 and has been accumulated and refined since 1964. During that period current data have been added to the historical files. Input is provided from personnel and accounting records, radiation monitoring systems, medical questionnaires, physical examinations, laboratory tests and the output of runs of personnel rosters through SSA with subsequent procurement of death certificates. Suitable error routines are used in the processing of the data, and provisions are made for confidentiality of the files of identified data.

Results of the analyses include the demonstration of a more marked healthy worker effect in long-term than short-term workers. Analyses of the relationship of radiation to mortality have disclosed no evidence of a positive trend of mortality with increasing radiation dose for all causes of death or for all cancer. A statistically significant trend has been observed for multiple myeloma and a questionable positive trend for cancer of the pancreas. No correlations are observed for myeloid leukemia or lung cancer. In interpreting these results, one must bear in mind that the analysis for trend was also conducted (Text Truncated -Exceeds Capacity)

63. ERDA HEALTH STUDY, CHRONIC RADIATION EXPOSURE - CORRELATION OF RADIATION EXPOSURE WITH BIOLOGICAL EFFECT

Kirklin, C. W., Hanford Environmental Health Foundation Inc., P.O. Box 100, Richland, Washington, 99352, United States of America

This project is to determine the long-range effect of low-level long-term occupational radiation exposure on man. The Department of Energy Richland Location (DOE-RL) Contractors employees' health, radiation exposure, and job assignment records have been compiled and organized in a data file dating from 1944. This, with other data, is being studied to develop relationships between radiation exposure and mortality. Data accumulation includes (1) employee identification data, (2) employment history, (3) sibling control groups, (4) occupational radiation exposure (internal and external), (5) medical x-ray exposure, (6) medical examination data, (7) environmental exposure other than radiation, and (8) mortality data.

64. ANALYSIS OF DATA FROM THE HANFORD MORTALITY AND MORBIDITY SURVEY (USA)

Stewart, A. M., Mancuso, T., University of Birmingham, P.O. Box 363, Birmingham, England, United Kingdom

Factors affecting the probability of workers in the radiation industry dying from malignant disease following occupational exposures to external radiation are being studied.

65. HEALTH AND MORTALITY STUDY

Lushbaugh, C. C., Dupree, B., Hansard, M., Frome, E. L., Oak Ridge Associated Universities Inc., Box 117, Oak Ridge, Tennessee, 37830, United States of America

Epidemiologic studies are being conducted on populations of DOE (or DOE contractor) workers whose occupations involve potential exposure to internal and/or external radiation. These workers also have the potential of being exposed to uranium, uranium dust, and such toxic metals as lead, cadmium, and nickel, etc., in nuclear fuel production and enrichment and in the processing of radioactive material related to military applications. The principal aim of these studies is to assess long-term health effects of occupational exposure to low-level ionizing radiation and additive effects by confounding chemical stresses. Current studies concern mortality experience of workers at sites in Oak Ridge (Y-12 Tennessee Eastman Corporation, Y-12 Union Carbide, X-10, and K-25 plants), other current

DOE facilities (Paducah, Portsmouth), disbanded Manhattan Engineering District (MED) sites (Mallinckrodt, Linde, Columbia University, Middlesex), and other selected sites (National Lead of Ohio, Mound, Savannah River, Argonne National Laboratory, Brookhaven National Laboratory, Idaho Test Site, and Nevada Test Site). Statistical methods and data-management techniques are being developed through methodology research for improving the power of epidemiologic analysis of low-level stresses to large numbers of people. This biostatistical research also provides record-linkage algorithms and statistical data-editing procedures to facilitate handling problems generic to using large complex data sets in longitudinal cohort studies. The long-range goal of this project is to be able to merge all occupational, rosters, radiation exposure records and other pertinent data from all MED/AEC/ERDA/DOE sites since 1942 into a National Atomic Energy Worker Roster to provide as many as possible person year rem's for radiation risk assessment and a basis for a continuous health and safety survey for DOE workers.

BIBLIOGRAPHIC REFERENCES: Armstrong, R.D., and E.L. Frome, Least Absolute Value Estimators for One-Way and Two-Way Tables, *Nav. Res. Logistics Quart.* 26:79-96 (1979). Armstrong, R.D., E.L. Frome and D.S. Kung, A. Revised Simplex Algorithm for the Absolute Deviation Curve Fitting Problem, *Commun. Stat.* 8B: 175-190 (1979).

66. HEALTH STUDY OF PLUTONIUM WORKERS

Voelz, G. L., Stebbings, J. H., Hempelmann, L. H., University of California, Central Office, U.S. Dept. of Energy Los Alamos Scientific Lab., Health Division, P.O. Box 1663, Los Alamos, New Mexico, 87545, United States of America

Past and present plutonium workers from six major United States nuclear installations will be followed to determine age-specific mortality rates from all major causes of death, with special emphasis on cancer. Dose-response relationships will be estimated using systemic body burden data from urine tests. The study population, approximately 20,000 individuals, will include all workers with estimated body burdens above one nanocurie, exposed workers with lesser or no body burdens, and random samples of non-exposed workers from the same installations. A cancer incidence study will be undertaken upon a subset of the study population; questionnaire studies of cardiovascular and chronic respiratory morbidity will also be carried out in that subgroup.

67. GENETIC EFFECTS, REPRODUCTIVE OUTCOMES, AND BIRTH DEFECTS AND OCCUPATIONAL EXPOSURE TO PLUTONIUM

Brown, S. M., Sandler, D., Hoel, D. G., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Environmental Health Sciences, Biometry Branch, P.O. Box 12233, Research Triangle Park, North Carolina, 27709, United States of America

The principal objective is to examine the reproductive outcomes principally of spontaneous abortion but also congenital defects and perhaps childhood cancer among a cohort of females associated with five federal facilities in which there was significant occupational exposure to plutonium. The work will be performed in two stages. In the first stage, a cohort study of female workers at the five facilities is entertained. The protocol of that study is currently being planned. The second stage would study the same outcomes in the wives or conjugal partners of exposed male workers at these same facilities. An attempt will be made to ascertain the existence of a dose-response relationship, at least for the most common outcome (spontaneous abortion).

68. HEALTH OF FORMER THORIUM WORKERS

Polednak, A. P., Brues, A. M., Rowland, R. E., Stehney, A. F., Argonne National Lab., Division of Biological & Medical Research, 9700 S. Cass Ave., Argonne, Illinois, 60439, United States of America

The study population is comprised of about 4000 persons (mostly males) who worked at a thorium processing plant from the mid 1930's until 1973. Mortality and causes of death (from death certificates) are being studied with internal comparisons (by job classification and length of employment) and external comparisons (using U.S. mortality rates specific for sex, age, time and cause). For morbidity studies, a subgroup of 558 males

(who worked for one year or longer in jobs probably involving highest exposure levels to thorium) has been selected, and questionnaires on medical and occupational history, respiratory symptoms and personal habits (smoking and alcohol consumption) have been sent to those not known to be dead. A random sample of 100 of these 558 men has been selected for a pilot study which involves measurement of radioactivity levels, and a medical examination (including pulmonary function tests). Interest in the health of thorium workers stems mainly from the potential importance of thorium as an energy source. Lung cancer and other lung diseases (e.g., pneumoconiosis) are of particular interest in these studies, since the major hazard involved in the thorium extraction process is believed to be related to inhalation of airborne thorium and thorium daughter products.

69. NATIONAL REGISTRY FOR RADIATION WORKERS

Reissland, J. A., Roe, S., Darby, S. C., National Radiological Protection Board, Dept. of Physics, Harwell OX11 0RQ, Didcot, United Kingdom

The purpose of this study is to investigate by long-term follow-up studies any relationship between occupational exposure to radiation and age-specific causes of death. Any worker who is monitored regularly for exposure to radiation and for whom dose records are kept is entered in the Registry. It is expected to include over 50,000 current workers in addition to those who leave the industry whether by death, retirement or change of job. Once sufficient data on mortality has accumulated, periodic analyses will be carried out with several different control groups chosen from among those workers on the Registry with very low lifetime radiation doses.

3. CANCER INCIDENCE AMONG OTHER WORKERS EXPOSED TO RADIATION

70. SPUTUM CYTOLOGY RELATED TO A FOUNDRY EXPOSURE

Simard, A., Band, P., Vauclair, R., Paquette, G., Saccomanno, G., Cole, P., Institut du Cancer de Montreal, Centre Hospitalier Notre Dame, 1560 Sherbrooke St. E., Montreal, Quebec, H2L 4M1, Canada

Some studies have shown an increased risk of lung cancer among foundry workers. Sputum cytology among uranium miners has shown cellular changes ranging from mild, moderate and marked atypia to cancer in-situ and invasive cancer. Sputum cytology will be undertaken in 700 foundry workers, using the technique of Saccomanno. Results will be related to duration of exposure in the foundry. Information on age, date of entry into the industry, work history, and smoking history will be obtained and person-years at risk evaluated. Comparisons will be made with other exposed groups, namely uranium miners, asbestos workers and smokers.

71. FOLLOW-UP ON RADIUM LUMINIZERS

Vennart, J., Baverstock, K. F., Medical Research Council, Radiobiology Unit, Harwell, Didcot, Oxfordshire OX11 0RQ, England, United Kingdom

There are two groups of luminizers, some with a record of body content of radium determined some years ago, and some without this measurement. Mortality data are being collected through national registration system.

72. EPIDEMIOLOGICAL ANALYSIS OF EFFECTS OF RADIUM

Rowland, R. E., Stehney, A. F., Chalfen, M. H., Argonne National Lab., Radiological & Environmental Research Division, 9700 S. Cass Ave., Argonne, Illinois, 60439, United States of America

Epidemiological studies are being conducted in order to determine the health effects of exposure to radium. Persons exposed to radium in various parts of the United States are being traced from records of radium dial companies and from other sources. Follow-up of persons located includes determination of vital status and analysis of causes of death (obtained from death certificates) for all, and medical examination, measurements of body radioactivity, and analysis of autopsy reports or materials for part of the group. Analyses of morbidity and mortality are made within the group in relation to body burden of radium. Dose-response patterns are being determined for bone sarcomas with reference to bone sarcomas and carcinomas

of the mastoids and the paranasal sinuses. Further analyses will involve other diseases. Mortality and morbidity in specific subgroups of radium cases (e.g., radium dial workers ascertained from employment lists) are being compared with U.S. statistics, with consideration of sex, and other demographic characteristics. At present, about 2,000 persons have been located and measured for radioactivity. Studies of the health of the offspring of certain groups (i.e., female radium dial workers) are also planned.

73. REPRODUCTIVE AND INDUSTRIAL HYGIENE STUDY OF WORKERS EXPOSED TO MW/RF ENERGY

Egan, B., Cox, C., Robinson, C., Herrick, R., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance Hazard Evaluations & Field Studies, 4676 Columbia Pkwy., Cincinnati, Ohio, 45226, United States of America

The potential biological hazards of exposure to radiofrequency radiation are not yet clearly understood and remain the subject of worldwide study. Radiofrequency radiation has been implicated in a variety of biological phenomena including altered transmembrane potentials, increased membrane permeability, hyperthermia, hormonal imbalance, chromosomal anomalies, and mutagenic and teratogenic effects. Most of these studies have been experimental in nature. Very few human studies have been conducted to evaluate the chronic or reproductive health effects associated with exposure to radiofrequency radiation. This lack of human data is especially true in the United States. Several unconfirmed allegations have been made associating exposure to radiofrequency radiation with pancreatic cancer, brain cancer, leukemia and an increased incidence of birth defects and miscarriages. Unfortunately, the size of the population potentially at risk in most of these case reports has been too small to conduct a scientific evaluation of the situation. It is the intent of this project to define a population of workers who have been employed in work areas where there is exposure to radiofrequency radiation in order to evaluate whether their employment resulted in any increase in reproductive problems. Walk-through surveys are being conducted in the efforts to identify a study population. To date, the plants have been found to employ predominantly women in the heat sealing area. A control population of comparable size will be selected from the same facilities as the study group, but should be employed in work areas where there is no potential for exposure to microwave radiation or other potentially hazardous exposures.

74. MORTALITY AND INDUSTRIAL HYGIENE STUDY OF SHIPYARD WORKERS EXPOSED TO RADIATION

Rinsky, R., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, National Inst. for Occupational Safety & Health, Division of Surveillance Hazard Evaluation & Field Studies, 4676 Columbia Pkwy., Cincinnati, Ohio, 45226, United States of America

In a proportional mortality study conducted by Drs Najarian and Colton, a significant increase in deaths due to neoplasms of lymphatic and hematopoietic tissues was reported among shipyard nuclear propulsion workers. The results of their study indicated that exposure to radiation at levels previously thought to be safe may be unsafe. The NIOSH subsequently initiated a retrospective cohort mortality study. As part of the study, approximately 27,000 civilian employees have been identified as having worked at this naval shipyard since 1954. To date, more than 3,000 deaths have been identified among the study population. The vital status will be determined for the remaining identified study population and death certificates retrieved and verified. Results of in-depth industrial hygiene and health physics surveys will be related to current and past occupational and chemical exposure to chemical and physical agents other than radiation. An exposure history will be compiled and assigned to appropriate job titles/locations.

75. MULTIPLE MYELOMA AND BRAIN TUMORS IN PHYSICIANS

Matanoski, G. M., Diamond, E. L., Elliott, E. A., Johns Hopkins University, School of Hygiene & Public Health, Dept. of Epidemiology, 615 N. Wolfe St., Baltimore, Maryland, 21205, United States of America

Studying the etiology of these two cancers and their correlation in physicians, a relatively homogenous population which is unique in having a high probability of exposure to repeated low-dose radiation and infections and good medical histories. The design is a case-control study with controls comparable by age, race, sex, and year of death selected from total physician deaths over fifteen years. An equal number of living controls will also be selected. Risk factors identified in previous studies will be examined by telephone interviews with next-of-kin, physicians associates, or physicians if a case-control. There will be special emphasis on determining the exact number of years of exposure to radiation and the estimated dose levels by year or dosimetry data. Questions concerning patient contacts, personal characteristics and medical history will be included. Information on residence, type of training and specialty are available from AMA records. A multi-variate analysis of data will be used to determine association of each of these cancers with radiation, infection, or other factors.

76. RISK OF CANCER IN X-RAY TECHNOLOGISTS

Schuman, L. M., University of Minnesota, Minneapolis Campus, School of Public Health, Dept. of Epidemiology, 1325 Mayo Memorial, Minneapolis, Minnesota, 55455, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

77. STUDY OF PARTICIPANTS AT NUCLEAR TESTS

Robinette, C. D., Jablon, S. N., National Academy of Sciences, National Research Council, 2101 Constitution Ave. N.W., Washington, District of Columbia, 20418, United States of America

Approximately 30,000 members of the US military who were present at atmospheric tests of nuclear weapons conducted in the 1950's and 1960's will be followed to determine if leukemia and other cancers known to be induced by radiation are occurring in excess. Participants will be asked about illnesses requiring hospital admission and reproductive history.

78. IDENTIFICATION OF HIGH-RISK OCCUPATIONAL GROUPS

Kessler, I. I., Mabuchi, K., University of Maryland, Baltimore Professional Schools, School of Medicine, Dept. of Epidemiology & Preventive Medicine, 655 W. Baltimore St., Baltimore, Maryland, 21201, United States of America

Evidence from the laboratory as well as from clinical observations and epidemiological studies is substantiating earlier findings on the role of occupational factors in human carcinogenesis. Recent clinical and epidemiological evidence relating environmental exposure to such materials as asbestos, vinyl chloride, radiation, etc. strongly suggests that a major effort should be undertaken to identify occupational agents which may be responsible for human cancer, parkinsonism and other chronic disease. An etiologically meaningful analysis of occupational histories must have at least two characteristics: first, the analysis must be conducted in such a fashion as to permit comparisons with the findings of previous investigators; and second, the analysis must make identification of potentially dangerous occupational exposures possible. To accomplish these dual purposes, two completely independent analyses of occupational data are being undertaken, the first in accordance with the United States Census Bureau Index of Industries and Occupations, making it possible to compare results with those of previous investigators who have used the same index, and the second classifying all occupational exposures judgmentally, for the purpose of identifying all possible potentially hazardous occupational or industrial categories. After identifying specific occupational hazards in this fashion, previous investigators could be contacted with a view to reanalyzing their data in terms of the potentially dangerous occupations or industries newly identified.

C. SKIN CANCER AND EXPOSURE TO SUN- LIGHT AND ULTRAVIOLET RADIATION

79. ENVIRONMENTAL FACTORS AND THE DEVELOPMENT OF SKIN CANCER

Cerimele, D. M., Cottoni, F., Retanda, G., Scuderi, N., Sanna, E., Bonu, G., Campus, G. V., Fois, G. M., Università degli Studi di Sassari, Dept. of Dermatology, Viale Mancini 5, 07100 Sassari, Italy

There is ample evidence that environmental factors and particularly prolonged exposure to the rays of the sun play a significant role in the development of skin cancer. This study intends to determine the actual incidence of skin cancers in Sardinia, and the degree to which environmental and constitutional factors are involved. A questionnaire, developed for the present study, collects information from skin cancer patients on place of birth, place of residence during last ten years, place of present residence, color of the skin, eyes and hair, capacity to tan and time of exposure to the sun. Patients treated are followed up in order to establish if people who develop skin cancer are prone to develop new cancers of the skin or inner organs in the future.

80. INCIDENCE OF SKIN AND LIP CANCER IN USSR, ROLE OF SOLAR ULTRAVIOLET RADIATION IN ETIOLOGY OF SKIN CANCER, MELANOMA AND LIP TUMORS

Guslitsa, L. N., Matveitshuk, Y. D., Kudriavtseva, L. Y., Podrushnjak, V. B., Academy of Sciences of the Ukrainian SSR, R. Kavetsky Inst. for Oncology Problems, General Mechanisms of Carcinogenesis, Vasilkovskaya Str. 45, Kiev 252127, Ukrainian S S R, Union of Soviet Soc. Republics

The main aim of this study is the establishment of incidence rates for skin and lip cancer in different regions and selected ethnic groups of the USSR. To determine the role of solar ultraviolet radiation in the causation of skin and lip cancer, correlation analysis of different climatic factors and the incidence of these cancers in the various republics and regions of the USSR will be undertaken. It is proposed to complete special questionnaires for patients with skin and lip cancer and appropriate controls. Using cancer incidence data for the most frequent sites, it is proposed to study time trends for the periods 1960-1975 for Ukrainian SSR and selected regions thereof.

81. SPECIAL NON-MELANOMA SKIN CANCER INCIDENCE SURVEY AND EPIDEMIOLOGY STUDY

Scotto, J., Fears, T. R., Wiser, H. L., Lisiecki, E. J., Radosevich, S. T., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Biometry Branch, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

This project, a collaborative effort by the NCI, EPA and other federal agencies, was initiated to provide more data relative to skin cancer and harmful solar ultraviolet. An urgent need for these data has existed since recent scientific reports have warned about the decomposition of stratospheric ozone by nitrogen oxides and chlorofluoromethanes (CFMs). Federal regulatory agencies have already recommended bans on the use of aerosol spray cans which use CFMs as propellants. In the near future, there will be critical reviews of all pertinent information by government as well as individual interests concerned with the threat of increased human skin cancer due to excess amounts of UV-B reaching the earth's surface, as its protective ozone shield is depleted. As mandated by Public Law 95-95 (amendment to the Clean Air Act of 1977), the NCI will continue its investigations by adding 2 new locations to its data bases during this fiscal year. This will provide more reliable national estimates of the degree of morbidity from nonmelanoma skin cancer as well as epidemiologic data on host and environmental factors, other than solar ultraviolet, associated with increased risk. New leads from 8 surveys just being completed will be pursued in two new surveys being implemented.

BIBLIOGRAPHIC REFERENCES: Scotto, J. and Fears, T.R.: Skin Cancer Epidemiology: Research Needs. In Proceedings of the International Conference on Ultraviolet Carcinogenesis. Natl. Cancer Inst. Monogr. 50: 169-177, 1979. Scotto, J. and Nam, J.: Skin Melanoma and Seasonal Patterns. Am. J. Epidemiol. 111: 309-314, 1980.

82. THE RELATIONSHIP OF UVB (ULTRAVIOLET) RADIATION TO CANCER OF THE SKIN IN MAN

Urbach, F., Machia, L. R., Berger, D. R., Robertson, D. F., Scotto, J., Temple University, School of Medicine, Dept. of Dermatology, 3322 Broad St., Philadelphia, Pennsylvania, 19122, United States of America

There is strong circumstantial evidence that skin cancer in man is causally related to chronic exposure to solar ultraviolet radiation. A series of automatic, integrating UVB meters has

been designed, built and installed in various locations. These meters measure sunburn-effective UVB continuously. Measurements of UVB are being compared to either incidence or prevalence of skin cancer in selected areas sufficiently differing in insolation in order to determine the relationship of UVB, stratospheric ozone concentrations and physical sunlight exposure to the induction of skin cancer in man.

83. EPIDEMIOLOGY OF MALIGNANT MELANOMA

Clark, W. H., Bernardino, E. A., Guerry, D., Hamilton, R., LaRosa, D., Bondi, E. E., Elder, D. E., Tuthill, R., Cassileth, B., Hospital of the University of Pennsylvania, Dept. of Dermatology, 3400 Spruce St., Philadelphia, Pennsylvania, 19104, United States of America

The objectives include a study of the role of sunlight in the aetiology and tumor progression of malignant melanoma. In addition, there are studies which will be related to family and ethnic factors in malignant melanoma.

84. ESTIMATING THE INCREASES IN SKIN CANCER THAT WILL RESULT FROM INCREASING ULTRAVIOLET RADIATION

Scott, E. L., Neyman, J., University of California, Berkeley Campus, School of Letters & Science, Dept. of Statistics, Berkeley, California, 94720, United States of America

Increasing ultraviolet radiation (from depletion of the stratospheric ozone or from other causes) will result in increasing skin cancer. The purpose of this study is to estimate the amount of the increase as a function of age, sex, locality, site, skin color, and other characteristics. Efforts are being made to ascertain the influence of microclimate, socioeconomic level, and other factors which may influence the observed increase in skin cancer. Separate studies are made for melanoma, non-melanoma, and keratosis in as much detail as possible; also for mortality, incidence, and prevalence.

85. THE IMPACT OF STRATOSPHERIC OZONE DEPLETION UPON TROPOSPHERIC ULTRAVIOLET, PHOTOCHEMISTRY AND SMOG

Green, A. E., Chamoides, W. L., State University System of Florida, University of Florida, Interdisciplinary Center for Aeronomy & Atmospheric Sciences, Gainesville, Florida, 32601, United States of America

OBJECTIVE: To attempt to determine if depletion of stratospheric ozone (O3) increases ultraviolet radiation at the earth's surface and to then examine possible impacts on skin cancer, plant yields and photochemical smog.

APPROACH: Undertake radiative transfer calculations and modeling studies of O3 depletion impact on tropospheric UV radiation and photolysis rates; and photochemical model calculations of the effect of enhanced UV radiation and photolysis rates on tropospheric photochemistry and smog formation.

PROGRESS: The investigators and others have previously done preliminary calculations leading to the hypothesis that there will be impact of O3 depletion on UV, photolysis and smog formation.

86. A STUDY OF INCIDENCE OF MALIGNANT MELANOMA IN IRELAND AND A STUDY OF ETIOLOGICAL FACTORS IN SKIN CANCER

Moriarty, M. J., Loughlin, G. O., Bourke, G. J., St. Luke's Hospital, Highfield Rd., Rathgar, Dublin 6, Ireland

OBJECTIVES: (1) To determine incidence of malignant melanoma in Ireland. (2) To investigate role of possible etiological factors: (a) UV exposure, (b) genetic factors - viz DNA repair, (c) immunological factors, (d) nutritional factors - viz vit. A plus lipid, (e) hematological factors.

APPROACH: (1) Review of all histology reports in the country for 2 yrs. (2) Case control study.

PROGRESS: Study started August 1979.

87. EPIDEMIOLOGY, PATHOLOGY, CLINICAL MANAGEMENT AND OUTCOME OF MALIGNANT MELANOMA IN WESTERN AUSTRALIA

Armstrong, B. K., Holman, C. D., Heenan, P. J., Hanson Knarchoi, H., University of Western Australia, Nedlands, W. Australia 6009, Australia

All patients (about 500 expected) having pre-invasive or invasive malignant melanoma of the skin diagnosed in Western

Australia in 1980 and 1981 will be included in a case-control study, and detailed review of histopathology and clinical management undertaken. The case-control study will include demographic features, details of sun-exposure (particularly intermittent versus continuous), objective measures of solar skin damage, diet and other relevant habits. Controls will be matched by age and sex using the electrical roll as a sampling frame. All relevant prognostic features will be recorded in the histopathological review including Clark's levels and tumor thickness. Melanoma patients will be followed for at least ten years to document prognosis in relation to histopathology and clinical management.

88. ANALYSIS OF COMPUTERIZED DATA ON MALIGNANT MELANOMA

Shaw, H. M., Milton, G. W., McCarthy, W. H., Hersey, P., University of Sydney, Dept. of Surgery, Sydney, N.S.W. 2006, Australia

A detailed study of malignant melanoma is being made from two points of view: factors in the host and tumor that influence the behavior of the tumor, i.e., disease-free periods and site and type of metastases when they develop. The factors being investigated from the existing records include: (1) identification of high risk individuals; (2) role of sunlight exposure. Studies from the United States and Europe indicate an inverse relation between incidence of melanoma and geographic latitude. However, there appears to be little correlation between morbidity and mortality. Because morbidity has increased more than mortality, there is a need to establish whether: (a) the increase in incidence is indeed a real effect or an artifactual one, resulting from earlier diagnosis and the more frequent removal of superficial tumors, and (b) melanomas induced by sun exposure have a lower degree of malignancy than those always covered by clothing; (3) tumor factors possibly unrelated to sunlight exposure. Morbidity may have increased more than mortality due to changes in the biological nature of the tumor, such as histological type, grade of mitotic activity, level of invasion or tumor thickness; (4) patterns of dissemination of melanoma. It is important to determine why melanoma spreads by either rapid hematogenous transmission, by slower direct lymphatic extension or extension through successive echelons of local and regional lymphatics.

89. CLINICOPATHOLOGIC CORRELATION BETWEEN DYSKERATOSIS AND SQUAMOUS CELL CARCINOMA OF THE CONJUNCTIVA

Arenas Archila, E., Amaya, L., Arenas Archila, M. A., Martin, F., Instituto Nacional de Cancerologia, Dept. of Oftalmologia Patologia, Calle 1 No. 9-85, Bogota, Colombia

OBJECTIVE: The high incidence of malignant lesions of the conjunctiva in close relationship with the ultraviolet radiation in countries near the Equator facilitates determining a clinicopathologic correlation in order to better manage this kind of progressive disease.

METHOD: A clinical photograph of each case is taken before surgery, and a complete histopathological study is performed. After all the cases with some clinical evolution are completed, a histological and clinical classification will be made. Finally, pathologists and ophthalmologists will produce a final classification in regard to management and prognosis.

PROGRESS: By now, we have collected about 50 documented cases which are in the process of histological review by two pathologists in different institutions.

90. ETIOLOGICAL FACTORS IN MALIGNANT MELANOMA OF THE SKIN

Adam, S. A., Vessey, M. P., University of Oxford, Dept. of Social & Community Medicine, S. Parks Rd., Oxford OX2 6HE, England, United Kingdom

A case-control study is being conducted to investigate the role of light, genetic factors, and certain drugs, including oral contraceptives, in the etiology of melanoma of the skin. Cases among women under 50 years of age are to be identified through cancer registries, hospital discharge records, and death certificates. The general practitioner of the case will be interviewed and three controls, matched for age, sex, and marital status, will be selected from his records. All cases and controls will be sent a postal questionnaire.

91. CASE-CONTROL STUDY OF MALIGNANT MELANOMA

Bahn, A. K., University of Pennsylvania, School of Medicine, Dept. of Research Medicine, 36th & Hamilton Walk, Philadelphia, Pennsylvania, 19174, United States of America

This is a two-year case-control study of 225 cases and matched controls resident in Northern New Jersey. All white males between the ages of 30 and 64 years in the selected counties with a diagnosis of malignant melanoma who were first diagnosed between July 1, 1978, and December 30, 1980, will be eligible as cases. Age-sex-race population matched controls will be selected.

Information obtained from records of private dermatopathology laboratories and hospital pathologist records will be used to determine cases aided by the New Jersey, New York and Pennsylvania cancer registers. All cases will be ascertained as malignant melanoma by a board certified dermatopathologist. Home interviewing of cases and controls will obtain information on: residence history, work exposure history (particularly to chemical substances), sun exposure, history of cancer in the family and history of chloracne dermatitis.

Preliminary work has provided suggestive evidence of a large relative risk of malignant melanoma among workers exposed to polychlorinated biphenyls (PCBs) and other chemicals. New Jersey has been selected as the locale for the study because this state has the largest number of workers in chemical and related industries in the nation and because high melanoma mortality rates (for an area outside the South) have been noted among males.

Investigation will be carried out to determine if there are significant differences between cases and controls in exposure to chemical substances, exposure to sunlight, or a previous history of chloracne dermatitis.

92. CASE-CONTROL STUDY OF MALIGNANT MELANOMA

Beral, V. S., Milton, G. W., University of London, London School of Hygiene & Tropical Medicine, Dept. of Medical Statistics & Epidemiology, Keppel St., London WC1E 7HT, England, United Kingdom

A case-control study of women aged 16-49 with malignant melanoma has been set up. 400 cases and 800 controls with be interviewed. The main aim of the study is to examine the relationship of oral contraceptive use to the risk of developing malignant melanoma. In addition, information about exposure to sunlight, occupation and reproductive history is being collected; the relationship of these factors to melanoma risk, and their inter-relation with oral contraceptive use will be examined.

93. MALIGNANT MELANOMA IN WOMEN ON CONTRACEPTIVE STEROIDS

Beral, V., University of Sydney, Sydney, N.S.W. 2006, Australia

OBJECTIVES: Characterize the association between use of oral contraceptives and occurrence of malignant cutaneous melanoma by a case-control study, determine relative risk, causative and confounding factors. Perform the following subsidiary analyses: The relative risk of melanoma associated with different durations of oral contraceptive use; the relative risk of melanoma associated with different formulations of contraceptives; the relative risk of melanoma at various anatomical sites associated with oral contraceptive use; the relative risk of different clinical stages and histological types of melanoma associated with oral contraceptive use; the relative risk of melanoma associated with estrogen hormone replacement therapy; the relative risk of melanoma among women using oral contraceptives who have intense sunlight exposure, compared with that in women who have little exposure to sunlight.

94. OCCUPATIONAL AND NON-OCCUPATIONAL RISK FACTORS OF SQUAMOUS CELL CARCINOMA OF SKIN IN THE MONTREAL REGION

Aubry, F., McGill University, Dept. of Epidemiology & Health, 3775 University St., Montreal, Quebec, H3A 2B4, Canada

Data from the Cancer Registry of the Province of Quebec indicate that skin cancer is one of the leading types of cancer in the Montreal region. Given that the two major risk factors of squamous cell carcinoma of the skin recognized in the medical literature are sunlight exposure and the Celtic characteristics of people and that these factors are not particularly marked in the

Montreal region, the possibility exists that other risk factors, particularly occupational, could play a role. The objective of this case-control study is to assess the relative risk associated with occupational and non-occupational known and suspected risk factors of squamous cell carcinoma of the skin diagnosed in the Montreal region, specifically occupational exposure to sunlight and/or to chemical agents and radiations. The cases are defined as all cases of squamous cell carcinoma of the skin diagnosed during the two years preceding the beginning of the study in the pathology departments of Montreal hospitals. Two controls matched for sex and age with each case will be selected from among pathology records at the same hospital at which the case was found. The controls will be subjects in whom a pathological diagnosis will have been made for skin condition which is non-malignant and which is not known to be related to the risk factors of squamous cell carcinoma of the skin. Cases of pemphigus vulgaris, bullous pemphigus, intradermal naevus and lichen planus will thus be included. The total number of cases in this study is estimated between 400 and 800. A postal questionnaire on occupation and exposure to environmental factors will be sent to each case and each control and telephone calls will then be made to complete missing information. Data will be analysed by means of a discriminant analysis.

D. ONCOGENESIS AND ENVIRONMENTAL EXPOSURE TO RADIATION

1. CANCER INCIDENCE AMONG NUCLEAR EXPLOSION SURVIVORS

95. THE LATE RADIATION EFFECTS OF ATOMIC BOMB EXPOSURE IN THE POPULATION OF HIROSHIMA AND NAGASAKI, JAPAN

Tamaki, M., Takabe, M., Schull, W. J., Ishimaru, T., Kato, H., Wakabayashi, T., Radiation Effects Research Foundation, 5-2 Hijiya Pk., Hiroshima 730, Hiroshima, Japan

The Atomic Bomb Casualty Commission (ABCC) in Japan was organized in 1947 under the supervision of the U.S. National Academy of Sciences (NAS) to detect late radiation effects in the people of Hiroshima and Nagasaki exposed to the 1945 atomic bombs. In 1975 ABCC was reorganized as the Radiation Effects Research Foundation (REFEF), a private non-profit foundation funded equally by Japan and the United States. The RERF offices, laboratories, and examination facilities are maintained in both Hiroshima and Nagasaki, where adult health examinations, autopsies, clinical research, and epidemiological studies are conducted. The basic population for the epidemiological and autopsy studies numbers about 109,000 survivors and their controls and 54,000 of their children. Biennial clinical examinations are conducted on about 15,000 survivors and their controls in the two cities. A number of important radiation-related clinical disorders and abnormalities have been detected in the atomic bomb survivors, including increased frequency of lenticular opacities, thyroid tumors, leukemia, chromosome aberrations in the peripheral lymphocytes, and slight impairment of growth and development of those exposed in utero, especially if significant exposure occurred during the early period of gestation. Recent findings indicate an increased incidence of solid tumors among the more heavily irradiated survivors most apparent for breast and lung cancers; this may extend to cancer of the stomach and other sites. The RERF plans to continue its health examination, mortality, and autopsy programs. Particular attention in the clinical examination population is now being focused on early cancer detection, cancer education, alteration of immunological competence, and acceleration of aging. The tumor and tissue registry programs in Hiroshima and Nagasaki complement the RERF studies for the detection of radiation-induced tumors.

96. LEUKEMIA IN ATOMIC BOMB SURVIVORS AND CONTROLS, HIROSHIMA AND NAGASAKI

Ichimaru, M., Ishimaru, T. A., Mikami, M. A., Matsunaga, M., Nagasaki University, School of Medicine, Atomic Disease Institute, 12-4 Sakamoto-Machi, Nagasaki, Nagasaki, Japan

The aim of this study is to examine radiation leukemogenesis in atomic bomb survivors paying attention to dose-response curve by gamma and neutron dose and the relationship between incidence rate, latency period, age at exposure and dose. The

occurrence of multiple myeloma is also being studied. A preliminary paper was published in the Journal of Radiation Research, 19(3): 262-82, 1978.

97. LEUKEMIA AND RELATED DISORDERS IN ATOMIC BOMB SURVIVORS

Ichimaru, M., Ishimaru, T., Nagasaki University, Atomic Disease Inst., Dept. of Hematology, Sakamoto-Cha, c/o School of Medicine, Nagasaki, Japan

Approximately 80,000 atomic bomb survivors and 30,000 controls are being studied. The radiation dose of each exposed person is calculated. 150 cases of leukemia and 22 cases of multiple myeloma have been confirmed in this sample from 1945-1976. The incidence of leukemia and related disorders, relationship to dose of gamma ray and neutron, chromosome anomalies, and differences between the two cities of Hiroshima and Nagasaki have been studied. A manuscript of a paper 'Multiple myeloma among atomic bomb survivors and controls in Hiroshima and Nagasaki, by dose, 1945-1976', was sent to the J. Nat. Cancer Inst. Follow-up continues.

98. CANCER MORTALITY AMONG ATOMIC BOMB SURVIVORS IN HIROSHIMA PREFECTURE

Kurihara, M., Munaka, M., Hayakawa, N., Hiroshima University, Research Inst. of Nuclear Medicine & Biology, Dept. of Epidemiology & Social Medicine, 2-3 Kasumi 2 Chome, Hiroshima 734, Hiroshima, Japan

The atomic bomb survivors in Hiroshima Prefecture were estimated in 1975 to be nearly 180,000. Using the data bank containing information about the survivors in our institute, a survey on cancer mortality among the survivors from 1965 onwards is being conducted. This includes comparison with mortality in the general population of all Japan and in the non-exposed population of Hiroshima Prefecture. The mortality statistics for 1968, 1969 and 1970 will be obtained in 1979.

99. STOMACH CARCINOMA IN A-BOMB SURVIVORS OF HIROSHIMA AND NAGASAKI, 1950-1977

Yamamoto, T., Matsuura, H., Radiation Effects Research Foundation, Dept. of Pathology, 5-2 Hijiya Pk., Hiroshima 730, Hiroshima, Japan

A histopathological review will be made of stomach carcinoma cases observed in a defined population in Hiroshima and Nagasaki during 1950-1977. This population, the Extended Life Span Study sample, consists of some 100,000 persons, of whom 3/4 were in the cities at the time of bombing, whose permanent address was in or near the cities. The sample includes about 9,000 persons who were less than 2.5 km from the hypocentre at the time of bombing. Autopsy and surgical material and the relationship of stomach carcinoma incidence and histological characteristics to A-bomb radiation dose will be investigated. This is an extension of a study published in J. Radiat. Res. 19,213-227, 1978.

100. DOSE REASSESSMENT FOR POPULATIONS ON RONGELAP AND UTIRIK FOLLOWING EXPOSURE TO FALLOUT FROM THE BRAVO INCIDENT (MARCH 1, 1954)

Naidu, J. R., Greenhouse, N. A., U.S. Dept. of Energy, Brookhaven National Lab., Upton, New York, 11973, United States of America

Incidence of thyroid nodules, benign and malignant, in the exposed populations of Utirik and Rongelap have indicated critical differences in correspondence between nodule incidence and thyroid dose for the two populations. The estimated external dose received from the time fallout began to the time of evacuation shows that the Rongelap population received an external dose (175 rads) which was about thirteen times that for the Utirik population (14 rads), and the thyroid dose was about ten times larger, whereas the incidence of thyroid nodules in the two populations were not significantly different. A preliminary study has indicated that the critical area of investigation that could shed light is the period during fallout and evacuation for both the islands. In addition, the fact that the Utirik population returned within 120 days following evacuation, whereas the Rongelap population returned only after three years, requires that we look closely at the Utirik population in terms of a longer period, both internal and external. Further studies would, therefore, have to concentrate on the re-examination of all avail-

able data in reports issued by various agencies during that period, consultations with uncertainty, and using appropriate computer programs to analyze the data. The end result will enable us to look for correlations between the incidence of thyroid nodules and the reassessed dose estimates.

101. MEDICAL EXAMINATIONS OF A MARSHALLESE POPULATION ACCIDENTALLY EXPOSED TO FALLOUT RADIATION IN 1954

Conard, R. A., Pratt, H., Brookhaven National Lab., Medical Dept., Upton, New York, 11973, United States of America

Medical findings including radiation effects are collected annually. Twenty-four years of study: benign and malignant thyroid tumors induced by radiation have occurred, as well as one case of acute myelogenous leukemia. Latest report: A twenty year review of medical findings in a Marshallese population accidentally exposed to radioactive fallout. Brookhaven National Laboratory (BNL) report 50424, 1975.

102. STUDIES OF CANCER AMONG ATOMIC BOMB SURVIVORS

Jablon, S., National Academy of Sciences, 2101 Constitution Ave. N.W., Washington, District of Columbia, 20037, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

103. EPIDEMIOLOGICAL STUDIES OF CANCER

Caldwell, G. G., Heath, J., Falk, H. L., Zack, M. M., Stein, G., Kreiss, K., Ing, R., U.S. Dept. of Health & Human Services, Public Health Service, Center for Disease Control, Bureau of Epidemiology, Cancer Branch, 1600 Clifton Rd. N.E., Atlanta, Georgia, 30333, United States of America

Various projects are being conducted: 1) A case-control study of interpersonal contact as a risk factor for leukemia/lymphoma in the Metropolitan Atlanta area; 2) laboratory epidemiological studies of families owning cats shedding feline leukemia virus (in collaboration with several tumor virus laboratories in the USA); 3) ad hoc field investigations of community clusters particularly leukemia/lymphoma; 4) retrospective assessment of leukemia risks among persons exposed to atmospheric atom bomb tests in the US in 1957 (in collaboration with other Federal groups); 5) prospective assessment of possible cancer risk in Michigan residents ingesting polybrominated biphenyl in 1973-74 (in collaboration with the State of Michigan and various Federal groups); 6) a follow-up study of women who have died of carcinoma of the cervix to determine whether or not the disease was potentially preventable.

2. OTHER STUDIES ON CANCER INCIDENCE AND EXPOSURE TO RADIATION

104. NATURAL BACKGROUND RADIATION AND CANCER FREQUENCY IN HUMAN POPULATION GROUPS

Allwright, S. P., Kevany, J. P., Doherty, M., McAulay, I. I., University of Dublin Trinity College, St. James Hospital, Dept. of Community Health, James St., Dublin 8, Ireland

Studies investigating the relationship between natural radiation and cancer have produced varying and inconclusive results. The objective of this study is to determine type specific age standardized cancer death rates in Ireland in relation to defined areas of high and low background radiation. Regions of probable high and low radiation have been selected on the basis of existing geological data. Radiation levels, both indoor and outdoor, in these areas will be monitored. The unit area for radiation monitoring will be ten sq km, based on the National Reference Grid. Cancer mortality in the regions where radiation has been measured will be determined, also based on the ten sq km unit of area.

105. STUDY OF NATURAL RADIATION

Masse, L. M., Tallua, B., Gallou, M., Ecole Nationale de la Sante Publique, Sect. de Statistique, Ave. du Prof. Leon Bernard, 35043 Rennes, France

The study aims to assess biological changes, including congenital malformations and cancer, occurring in a rural population of 2,400,000 with a relatively high level of background radiation. Within this population 120,000 are particularly highly

exposed and mortality rates for this cohort are being compared to those of the general population of Brittany and of the Republic of Ireland.

106. BACKGROUND RADIATION AS A CARCINOGENIC HAZARD

Frigerio, N. A., Stowe, R. S., Argonne National Lab., Radiology Group, 9700 S. Cass Ave., Argonne, Illinois, 60439, United States of America

OBJECTIVES: To compare cancer incidence and mortality rates with background radiation levels in various regions of the world. Comparisons include components of background radiation, and all the various types of malignancies and anatomical sites.

107. EVALUATION OF LOW LEVEL PLUTONIUM AND CANCER

Johnson, C. J., Jefferson County Health Dept., 260 S. Kipling St., Lakewood, Colorado, 80226, United States of America

Because of documented contamination of a large area of land with plutonium and other transuranic radionuclides in the vicinity of the Rocky Flats nuclear weapons plant in the Denver Standard Metropolitan Statistical Area, there is community concern regarding possible health effects in populations living in this area as well as concern for the safety of further residential development near the plant. A preliminary study of age-corrected cancer death rates in eight contaminated census tracts around the plant compared with a control area of 19 census tracts with a similar population in a relatively uncontaminated part of the S.M.S.A. disclosed a significant increase in deaths from Leukemia and lung cancer in the contaminated area. The study has been expanded to evaluate incidence and mortality rates of leukemia, lung cancer, and other types of cancer in census tract areas selected by concentrations of plutonium in soil or in surface respirable dust. The first phase of the study is the evaluation of cancer incidence in the contaminated and uncontaminated census tract areas of the S.M.S.A., utilizing data from the Third National Cancer Incidence Survey of 1969-71 by the National Cancer Institute. Cancer incidence in the contaminated areas, corrected for age, sex, race, ethnicity, income and education, will be compared to that in the uncontaminated census tract area of the S.M.S.A. by several methods, including regression analysis. The second phase is the evaluation of cancer death rates between 1970 and 1980, and the third phase will be an analysis of cancer incidence in the period 1979-81, using the same study and control areas as in the first phase. Cancer death and incidence data will be supplied by the Colorado Health Department, Division of Vital Statistics and the Colorado Cancer Registry. The effects of exposure to small concentrations of plutonium and other transuranics on a general population living in a contaminated area has never yet been evaluated with this epidemiologic approach, and the results will be of considerable value in selecting sites for plutonium processing plants and nuclear-electric generating plants in relation to population centers.

This study will be followed by a prospective study extending to 1991 (Phase four).

108. EPIDEMIOLOGICAL STUDY OF LEUKEMIA IN MESA COUNTY, COLORADO, U.S.A.

Ferguson, S. W., Franz, L., Vernon, T., Cunningham, M., State Div. of Epidemiology & Disease Control, 4210 E. 11th Ave., Denver, Colorado, 80216, United States of America

This is a descriptive and analytical study in Mesa County, Colorado, USA, where the age-adjusted leukemia incidence rates are 2-2.5 times the expected, based on Colorado and US data (41 observed: 20 expected in 1970-1976). In the 1950s and 1960s tailings for a uranium mill were used extensively in Mesa County for construction - as a backfill under basements and around foundation walls. For the analytical study the case-control approach is used. Leukemia patients, patients with other malignancies believed associated with ionizing radiation and patients with cardiovascular disease are interviewed. Work over the past year has confirmed the leukemia excess, but has not shown a direct association with residences containing uranium mill tailings.

109. THE EFFECTS OF LOW LEVEL GAMMA AND ALPHA RADIATION ON PERIPHERAL BLOOD LYMPHOCYTE CYTOGENETICS AND SPUTUM CYTOLOGY

Ferguson, S. W., Franz, L., Hopkins, R., Ouimette, D., Hamman, R., Brandom, W., Saccomanno, G., State Div. of Epidemiology & Disease Control, 4210 E. 11th Ave., Denver, Colorado, 80216, United States of America

The aim is to study the effects of low level gamma and alpha radiation (radon and radon progeny) upon lymphocyte cytogenetics and sputum cytology among 150 long-term residents of Grand Junction, Colorado, compared to 150 long-term residents of Greeley, Colorado. Construction of homes, schools, etc. in Grand Junction from 1957 until 1966, using uranium mill tailings, exposed many persons to radiation significantly above background level. Greeley is a control city where mill tailings are not used in construction.

110. LUNG CANCER - SMOKING, RESIDENCE AND WORK PLACE EXPOSURES

Edling, C., Axelsson, O., Sederholm, C., University of Linköping, University Hospital, Dept. of Occupational Medicine, 58185 Linköping, Sweden

Radon concentrations are expected to increase in dwellings due to decreased ventilation for energy saving. Estimates by the Swedish Energy Commission indicate that radon and radon daughters in dwellings could contribute considerably to lung cancer morbidity. Recently a case-control study from an extremely rural area indicated an increased risk of lung cancer among residents in stone houses (i.e. the higher concentrations of radon and daughters) in comparison to those who had lived in wooden houses (low concentrations) (Scand. J. Work Environ. Health, 5, 10-15, 1979). Another case-referent study has started to examine the hypothesis that indoor concentrations of radon and daughters are of etiological importance for lung cancer. Type of residence, work place exposures, smoking, etc. are examined in 120 newly diagnosed lung cancer cases and 480 matched controls selected from hospital registers. If earlier results are confirmed, efforts to save energy through decreasing ventilation mean a serious lung cancer threat to the populations in countries with a cold climate. However, if this hazard can be more definitely assessed, indoor air could be filtered to remove radon daughters.

111. CARCINOGENIC IMPACT OF RADON IN WATER SUPPLIES

Prichard, H. M., Mendez, S., University of Texas, Health Science Center at Houston, School of Public Health, Dept. of Environmental Science, 6515 Freeman, Houston, Texas, 77025, United States of America

The goal of this project is to evaluate the feasibility of an eventual epidemiological test of the linear, non-threshold model of radiation carcinogenesis using health effects data from communities with populations exposed to varying levels of Radon-222 in the water supplies. To accomplish this goal, two sub-projects must be carried out concurrently: 1. The establishment of a data base consisting of radon levels in selected community water supplies; 2. The development of an algorithm which produces a reasonable estimate of the community population dose increment as a function of practical variables such as radon content of the water supply, meteorological data, and local demographic and architectural factors.

Data for the first sub-project is being collected by a large-scale systematic sampling program which makes use of mail-out sampling kits and a simplified analysis technique developed in this Department. It is projected that by the end of the project, the water supplies of communities containing up to 15 million inhabitants can be characterized.

Laboratory and field experiments are being conducted to determine radon levels, radon progeny equilibrium status, unattached fraction, and other dose modifying factors in the indoor environment. Other experiments seek to document the relationship of indoor radon levels to water consumption and radon content of the water.

112. COMPARE OCCURRENCE AND LEVELS OF DRINKING WATER CONSTITUENTS AMONG 12 MATCHED PAIRS OF HIGH AND LOW CANCER MORTALITY COUNTIES (ABBREV)

Greathouse, D. G., Michael, G., Kraemer, D., U.S. Environmental Protection Agency, Office of Research & Development, Health Effects Research Lab., 3555 Ridge Ave., Cincinnati, Ohio, 45268, United States of America

OBJECTIVE: The aim of this project is to determine if matched counties with contrasting cancer mortality rates use drinking water that differs significantly in terms of occurrence and levels of contaminants, treatment technologies used, and/or source type (ground or surface).

APPROACH: Twelve pairs of matched counties were selected; matching criteria were region of the country, avg. educational level, percent of the population employed in the manufacturing industry, percent urbanization, total population years at risk. Within each pair, one county had a high and the other a low mortality rate for females due to colon, rectum, bladder, and liver cancer. Historical water quality and treatment information were collected for performance. Extensive water analyses were performed; analysis included determinations for 40 inorganics, trihalomethanes, TOC, fluorescence, asbestos, radionuclides, and mutagens.

PROGRESS: The field work has been completed and all water samples have been analyzed. Most of the data has been computerized. It is anticipated that the data analysis effort will begin by October 1, 1979 and be completed by March 31, 1980.

113. GEOGRAPHICAL DISTRIBUTION OF MALIGNANT NEOPLASMS OF THE BRAIN IN CANADA

Neutel, I. C., University of Ottawa, Faculty of Medicine, Dept. of Epidemiology & Community Medicine, 1145 Carling Ave., Lady Grey Bldg., Ottawa, Ontario, K1Z 7K4, Canada

Malignant neoplasms of the brain have received very little epidemiological attention in spite of the low survival rate and their occurrence in childhood or early adulthood. A few studies dealt with their general distribution in the United States and Denmark and only one studied the geographical distribution in relation to environmental factors. This project will study the geographical distribution of brain tumours across Canada using both mortality and incidence data by calculating age-adjusted mortality and incidence rates for census divisions. These rates can be related to various environmental variables known for the census divisions. In addition, this study will investigate the occupations recorded for a select group of brain tumour deaths and compare them with a matched group of controls. Of special interest will be occupations likely to involve chemical or radiation exposure. Prenatal radiation has been shown to increase the risk of childhood cancer of the central nervous system, and the same situation may apply to adult brain cancer.

114. EPIDEMIOLOGICAL STUDY OF CEREBRAL TUMORS

Burch, J. D., Howe, G. R., Miller, A. B., National Cancer Inst. of Canada, Epidemiology Unit, 77 Bloor St. W., Toronto, Ontario, M5S 2V7, Canada

OBJECTIVE: The study is designed to investigate the relative contribution of the known and suspected aetiological factors to the incidence of cerebral tumors in adults. The study will investigate factors such as urban/rural differences, occupation with specific reference to farming, the rubber industry, and exposure to vinyl chloride, familial associations, ionizing radiation, brain trauma, and specific chemical carcinogens. These associations are weak and the study will re-investigate them and explore other possible relevant factors such as exposure to nitrosamines especially through drinking water and preservative containing meats. A number of questions will also be asked of respondents relating to additional environmental factors e.g., smoking, alcohol consumption, etc.

APPROACH: The study is a case/control study with data on past events and exposures obtained from cases and hospital controls through personal interviews in the respondents' homes, using proxies for dead or incapacitated cases and appropriate proxy controls. Case acquisition is restricted to Metropolitan Toronto and southern Ontario and will include incident cases with defined histology from January 1, 1977, to December 31, 1980.

Hospital controls will be matched to cases by sex, age (as near as possible), marital status, and date of admission to hospital for live cases, date of death for deceased cases. Hospital controls will be patients admitted to hospital with any condition other than tumor of any site.

115. CANCER INCIDENCE AND EXPOSURE TO NATURAL IRRADIATION

Masse, L. M., Pincet, J., Gallou, M., Tallua, B., Ecole Nationale de la Sante Publique, Dept. of Statistique, Ave. du Prof. Leon Bernard, 35043 Rennes, France

Incidence of cancer by village according to the level of natural irradiation.

II. EXPERIMENTAL RADIATION CARCINOGENESIS

A. ULTRAVIOLET RADIATION CARCINOGENESIS

116. ULTRAVIOLET RADIATION CARCINOGENESIS

Fry, R. J., U.S. Dept. of Energy, Oak Ridge National Lab., P.O. Box X, Oak Ridge Operations Office, Oak Ridge, Tennessee, 37830, United States of America

OBJECTIVES: 1. To develop mouse model systems for the study of ultraviolet radiation (UVR) carcinogenesis and some of the factors that influence carcinogenesis of the skin in particular, and carcinogenesis in general. 2. To investigate the differences in susceptibility for UVR carcinogenesis that are dependent on genotype. These latter studies are designed to capitalize on the differences between, and the similarities in, two stocks of hairless mice, namely SKH:hairless-1 and HRS/J/An1.

MAJOR FINDINGS: Mutant mouse stocks have been selected containing four autosomal mutations, all of which result in a hairless phenotype but exhibit differences in the presence of sebaceous glands, the thickness of the skin and amount of residual follicular tissue. These mutations have been given the following provisional names: poor fur, fur loss, near naked and bald. There has been good progress in backcrossing the selected mutants to the two inbred strains - BALB/c and C57BL/6. In the case of BALB/c all four mutants are in the second backcross generation and in the third backcross generation with the C57BL/6, except for the near naked which is in the fourth backcross generation. The fertility in all backcrosses has been quite satisfactory with an average litter size of more than six and with a satisfactory number of mutant mice weaned. Previously it was found that SKH:hairless-1 and HRS/J/An1 mice differ in their susceptibility for skin carcinogenesis. Their immune competence has now been examined by using the following assays: 1. The proliferation of spleen T cells stimulated with PHA and Con A and LPS as assayed by 3H-thymidine incorporation showed only subtle differences between strains and age groups. There was no indication of immunological deficiency based purely on this assay. 2. Delayed hypersensitivity, as measured by the footpad swelling technique, was found to be less in SKH:hairless-1 and haired HRS/J/An1 mice than in hairless HRS/J/An1. 3. In vitro antibody synthesis was found to be lower in HRS/J/An1 than in SKH:hairless-1 mice. 4. The lymphoproliferative response was significantly less in SKH:hairless-1 mice than HRS/J/An1 mice. (Text Abridged.)

117. RELATIONSHIP OF DOSE AND EFFECT IN CARCINOGENESIS BY UV RADIATION

van der Leun, J. C., de Gruijl, F. R., van der Meer, J. B., Rijksuniversiteit te Utrecht, Inst. of Dermatology, Catharijnesingel 101, 3500 CG Utrecht, Netherlands

OBJECTIVE: The aim of this investigation is to find a correct description of the dose-response relationship in carcinogenesis by ultraviolet radiation. This will be used in the first instance for a better-founded estimation of the impact of a possible decrease in stratospheric ozone on the incidence of skin cancer.

APPROACH: Groups of albino hairless mice are exposed daily to the radiation from fluorescent sunlamps. Various groups of mice are given different daily doses of UV radiation; the doses given extend to lower, more realistic, values than in earlier investigations. The tumors formed are counted and exam-

ined histologically. By combining these and other experimental results with epidemiological data, an attempt is made to develop a consistent model for carcinogenesis by UV radiation.

PROGRESS: The first groups of mice have been under exposure since July 1, 1978. In the meantime we developed, on the basis of experimental and epidemiological data available, a theoretical relationship between dose and incidence of UV-induced skin cancers in a human population. It yields an over-all amplification factor of 4, i.e., a decrease in stratospheric ozone of 1% would ultimately lead to an increase in skin cancer incidence of 4%.

118. SKIN CARCINOGENESIS

Hashimoto, K., Kumakiri, M., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Dermatology Section, 4100 W. 3rd St., Dayton, Ohio, 45428, United States of America

The purpose of this study is to find specific UVA spectra which most effectively produce skin cancers. The Acridine Orange method developed by Kumakiri and Hashimoto (J. Natl. Cancer Inst 59:755-785, 1977) was used to monitor carcinogenesis in nude mice.

Several nude mice were irradiated with UVA produced squamous cell carcinoma, grade II and grade III. Acridine orange staining was used to compare the grain number between normal, precancerous and grade II and grade IV squamous cell carcinomas. Experiments using monochromatic UVA will be initiated upon receipt of a JASCO Spectro irradiator.

119. HAIRLESS MICE FOR UV CARCINOGENESIS STUDIES

Forbes, P. D., Temple University, School of Medicine, Dept. of Dermatology, 3322 Broad St., Philadelphia, Pennsylvania, 19122, United States of America

OBJECTIVES: Determine the comparative UV photocarcinogenic sensitivity of selected stocks and strains of nude mice. Characterize several immunologic traits of the selected animals. Evaluate breeding efficiency of stocks or strains which might be used as preferred test organisms for photocarcinogenesis.

120. ULTRAVIOLET RADIATION-INDUCED SKIN CANCER IN EXPERIMENTAL ANIMALS

Urbach, F., Temple University, School of Medicine, Dept. of Dermatology, 3322 Broad St., Philadelphia, Pennsylvania, 19122, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

121. UV-X-RAY INTERACTION - MUTATION AND TRANSFORMATION

Han, A., Elkind, M. M., University of Chicago, U.S. Dept. of Energy Argonne National Lab., Division of Biological & Medical Research, 5801 S. Ellis Ave., Chicago, Illinois, 60637, United States of America

Interaction of damage from nonionizing and ionizing radiations has been studied in a number of biological systems, but most extensively in microorganisms. Synergistic action has been shown in cell killing and in induction of mutations. In Chinese hamster cells, however, the damage from the two radiations is not synergistic. When doses of each radiation producing equal levels of survival are given immediately after each other in the middle of the DNA synthetic phase, where the greatest interaction takes place, cell killing from X-ray damage is completely additive to the far-UV sublethal damage. However, far-UV damage is only in part additive to sublethal damage induced by X-rays. When an interval for repair is introduced between the two radiations, the loss of interaction was more rapid after an initial dose of X-rays than after an initial dose of far-UV. These observations could be relevant to other end points like mutation and neoplastic transformation.

We propose to use mammalian cells in culture, Chinese hamster and mouse embryo C3H/10T1/2 cells, to determine the modifications in the frequency of mutations and neoplastic transformation due to far-UV and X-ray damage interaction. The effect of dose fractionation of single and combined treatments will be determined, and the effects obtained will be analyzed relative to the known repair processes in mammalian cells. If DNA damage is involved in induction of mutation and/or neoplastic transformation in a way similar to the DNA damage leading to cell lethality, combined treatments with cytotoxic

agents may yield frequencies different from those predictable on the basis of independent action.

122. TRANSFORMATION OF 3T3 CELLS BY ARTIFICIAL LIGHT SOURCES

Withrow, T. J. U.S. Dept. of Health & Human Services, Public Health Service, Food & Drug Admin., Bureau of Radiological Health, Experimental Studies Branch, 5600 Fishers Ln., Rockville, Maryland, 20857, United States of America

A number of artificial light sources will be evaluated for carcinogenic potential by means of an *in vitro* test system. These light sources will be studied for their toxic effect on cells, and for their effect on the transformation of cells in culture. The criterion for transformation will be the ability of the cells to grow in agar, which correlates well with the ability of the cells to grow as tumors when injected into an isologous host. Mouse (3T3) cells will be used for the transformation and toxicity studies. The cells will be exposed to a light source for varying amounts of time, grow in culture for 1 week to allow for expression, and then tested for the ability to grow in agar.

123. UNIVERSITY OF COPENHAGEN PROJECT

Ebbesen, P., Due, K., Olsson, L., Kobenhavns Universitet, Dep. of Tumor Virus Research, Frue Plads, 1168 Copenhagen, Denmark

We have previously demonstrated that senescent mouse skin, even when growing on a young syngeneic recipient, is characterized by A) an increased susceptibility to skin tumor induction with DMBA and beta-irradiation, B) a decreased content of chalone-like material, C) unresponsiveness to the effect of i.p. injected chalone-like material, and D) (in one experiment) development of more leukemias after DMBA painting of the senescent skin than after treatment of younger skin.

We now propose to study further virologic, immunologic and chalone mechanisms that could play part in bringing about the modifying effect that skin senescence has on skin tumor development after DMBA treatment; furthermore, to test if senescent skin also has enhanced susceptibility to the skin tumor-inducing effect of UV-light, if UV-irradiation can induce leukemia and if it can be confirmed that skin senescence enhances the leukemia inducing effect of DMBA treatment of the skin.

124. MECHANISM AND PREVENTION - LONGWAVE UV CARCINOGENESIS AND PHOTOAUGMENTATION

Willis, I., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Dermatology Service, 1670 Clairmont Rd., Decatur, Georgia, 30033, United States of America

The specific aims of this investigation are: (1) to determine what abnormal histological, electron-microscopic, physiological, and biochemical changes occur in skin after single and repeated UVA and visible light exposure and whether responses to combination of these wavelengths with UVB may differ from effects due to UVB alone. In addition, further objectives are to: develop new methods and materials and agents to protect against photoaugmentation and guidelines concerning potential health hazards that may result from excessive natural and artificial UVA and visible light exposure, and to elucidate the mechanisms of UVA photoaugmentation in its role in the induction of actinic malignancy, and reevaluate certain current methods of therapy for actinic cancers.

It is of special interest that after 5 years of chronic high dose UVA and UVA plus UVB exposures to the same test sites there is no evidence of cancerous changes in the skin. However, we have noted significant histological changes that are characteristic of severely actinically damaged skin. In our most recent studies we have concluded that the acute effect of irradiation with UVA is stronger and deeper than that of UVA-UVB combinations when biologically equivalent amounts of these radiations are delivered to the skin (1 MED doses). A most significant finding on electron microscopy was the destruction of Schwann cells and perineural cells in the skin following acute exposures. However, after long-term repeated exposures many free nerve endings were found in the dermal epidermal junction above the basal lamina and some of them made terminal enlargements. Dermal free nerve endings were also increased in number and some were situated below the melanocytes. There was multiplication of basal lamina and amorphous material was precipitated around the non-myelinated Schwann cells. Since a

multiplication of basal lamina may suggest a degeneration of the cells at the site of injury in the nervous system, cutaneous nerves were probably damaged by UVA and then regenerated. Production of amorphous substance seems to protect the nerves from repeated UVA exposures.

125. PHOTOAUGMENTATION AND CARCINOGENESIS

Willis, I., Israilli, Z. H., Emory University, School of Medicine, Dept. of Dermatology, 1364 Clifton Rd. N.E., Atlanta, Georgia, 30322, United States of America

Results of studies during the past year utilizing a newly developed tumor induction technique in the hairless mouse have revealed certain extremely important data which include the induction of spindle-celled squamous cell carcinomas that histopathologically most closely resemble a Grade 4, highly malignant, metastasizing form of squamous cell carcinoma usually seen primarily in areas of radio-dermatitis in human patients. Current studies are underway to differentiate the induction and progression of these tumors from those of the routinely induced Grade 2 to 3 type tumors during the application of this technique. Concurrent studies will include prophylaxis and treatment of both types of tumors.

Developments are underway to develop a sunscreensing formulation that will prevent the photoaugmentation effect of UVA on UVB irradiated skin. This will require the formulation of substantive chemicals that meet all of the criteria as outlined in the Federal Register on Sunscreens of 1978.

Results of studies to be conducted during the ensuing year will provide further insight into the induction of squamous cell cancer and the proper development of prophylactic agents and treatment measures.

126. PHOTOAUGMENTATION AND CARCINOGENESIS

Willis, I., Israilli, Z. H., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Dermatology Service, 1670 Clairmont Rd., Decatur, Georgia, 30033, United States of America

The overall objective of this proposal is to use a highly predictable *in vivo* animal model, developed in our laboratories, for the rapid induction of squamous cell cancer by UVB light in order to 1) gain better insight into the pathogenesis and treatment of squamous cell cancer, 2) develop a useful *in vivo* system for more rapid evaluation of cancer chemotherapeutic and immunotherapeutic drugs, and 3) develop more effective sunscreens, drugs and agents.

Studies using BCG immunotherapy revealed striking results in that in 80% of animals treated with 0.5 ml, 1.5 to 2.3 x 10 to the 7th power CFU injected under the base of the tumors, tumors began to regress fairly rapidly. However, in 20% of the animals there was initial partial regression and then subsequent accelerated growth. Since all tumors had been judged as clinically similar prior to initiation of therapy one would expect all tumors to respond similarly. To our amazement tumors that did not respond to immunotherapy were histopathologically spindle-celled squamous cell carcinomas that closely resembled fibrosarcomas. Using our system of tumor induction we had not previously encountered grade IV type squamous cell carcinomas although the tumors did eventually lead to death in all animals. Current studies are underway to determine a BCG dose schedule and chemotherapy that might be effective in the treatment of these tumors and to determine what led to their development during the course of irradiation.

127. PHOTOCHEMICAL REACTIONS RELATED TO SKIN CANCER

Black, H. S., Baylor College of Medicine, School of Medicine, Dept. of Dermatology, 1200 Moursund Ave., Houston, Texas, 77025, United States of America

The role of ultraviolet light (UVL) and sunlight in the etiology of certain types of skin cancer seems well established. The mechanism(s) associated with these processes are not understood. It has been suggested that the carcinogenic activity of light is dependent upon the photochemical conversion of sterols to carcinogenic substances. The formation of a sterol-derived photoproduct with carcinogenic properties has now been demonstrated in UV-irradiated human and hairless mouse skin. The role of this compound, cholesterol alpha-oxide, in the etiology of UV-carcinogenesis is under investigation.

This proposal is designed to determine whether additional information will implicate the compound in the etiology of UV-carcinogenesis and involves: 1) Studies on the role of antioxidants in suppression of UVL-carcinogenesis; 2) studies on the metabolism and detoxification of the carcinogen; 3) the ability of the carcinogenic compound to transform normal cells, in cell culture, into cells with malignant potential; and 4) the ability of the carcinogen to induce tumors when applied, in vivo, to mice. Should the carcinogen induce transformation in cell culture and initiate tumors in vivo, then an overwhelming body of evidence implicating the compound in the etiology of skin cancer will have been amassed. Studies of the metabolism of this compound may indicate means by which its formation can be prevented.

BIBLIOGRAPHIC REFERENCES: Black, H.S., C.M. Kleinheins, H.T. Hudson, R.M. Sayre, and P.P. Agin. Studies on the mechanism of protection by butylated hydroxytoluene to UV-radiation damage. *Photobiochem. Photobiophys* 1:119-123, 1980.

B. IONIZING RADIATION CARCINOGENESIS

128. RADIATION TOXICITY IN DOGS

Fritz, T. E., U.S. Dept. of Energy, Argonne National Lab., 9700 S. Cass Ave., Argonne, Illinois, 60439, United States of America

The basic objective of this program is the generation of comprehensive data on the late effects of low doses of ionizing radiation in a large, long-lived animal, the dog, that will be useful for extrapolation to man. The specific goals and areas of investigation are: (1) to determine the influence of three major factors of exposure - (a) the daily exposure rate, (b) the accumulated total exposure, and (c) the influence of age at the time irradiation is initiated; (2) to provide data for estimates of radiation-specific excess mortality in the dog in order to allow interspecies comparisons to existing rodent data and for development of a unifying concept of damage that has predictive value in man; and (3) to provide base-line data and specimens to characterize the pathogenesis and mechanisms of induction of leukemia and other disease processes. These experiments utilize protracted whole-body ⁶⁰Co gamma-irradiation given 22 hours/day, 7 days/week. They are characterizing the responses at various exposure rates given: (1) until death (beginning as young adults); (2) until predetermined total exposures of irradiation (beginning as young adults) are accumulated; and (3) during various stages of development and growth.

BIBLIOGRAPHIC REFERENCES: Fritz, T.E., Canine Enteritis Caused by a Parvovirus-Illinois, *J. Am. Vet. Med. Assoc.* 174: 3, 6 (1979).

129. CELLULAR FINE STRUCTURE AS A SENSITIVE INDICATOR OF ALTERED CELL FUNCTION IN CHRONIC TOXICITY STUDIES

Seed, T. M., U.S. Dept. of Energy, Div. of Biological & Medical Research, 9700 S. Cass Ave., Argonne, Illinois, 60439, United States of America

Research objectives include: (1) the clarification and description of the progressive development of aplastic anemia and leukemia in large, long-lived animals (e.g., dog) following protracted exposure to low daily doses of ionizing radiation or selected chemical toxicants (e.g., benzene); (2) the provision of sensitive and reliable predictors, based on early cellular events, of such pathological responses; and (3) the establishment, for benzene and gamma radiation, of the minimal toxic dose rates and exposure durations for pathological potential in the most sensitive target cells and tissues at risk. The experimental approach is to serially assess, in the exposed animal, phase-related changes in blood forming tissues. Detailed examination is being carried out of the various cellular compartments of the hematopoietic system, with particular reference to the structural and functional properties of stem cells; i.e., suspected cellular targets for these toxicants. To date, four distinct preclinical phases in leukemia induction have been described. Further work promises to elucidate specific cellular events, based on target-stem cell responses to the toxicant, reflective of the extent of pathological progression.

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sequences of Protracted Gamma Irradiation in the Beagle: Pre-clinical Phases of Leukemia Induction, Proceedings of the International Atomic Energy Agency Symposium on the Late Biological Effects of Ionizing Radiation, Vienna, March 13-17, 1978, in press.

130. TUMOR INCIDENCE IN GAMMA-IRRADIATED RABBITS

Hulse, E. V., Walding, P., Medical Research Council, Radiobiology Unit, Harwell, Didcot, Oxfordshire OX11 0RQ, England, United Kingdom

OBJECTIVE: To study the effects of dose rate on the incidence of tumours in gamma-irradiated rabbits.

APPROACH: Adult Dutch rabbits are exposed to whole-body doses of gamma-rays (100-1000 rad) at 3 dose rates differing by factors of 10. The rabbits and non-irradiated controls are kept for their natural life span, i.e., up to just over 9 years. The investigation is an extension of a study of tumor incidence after neutron or gamma irradiation (preliminary report: *Int. J. Radiat. Biol.*, 16, 27 (1969) and final report in preparation).

PROGRESS: The most frequently encountered tumors are basal-cell tumors of the skin, fibrosarcomas, osteosarcomas and Sertoli cell tumors of the testis.

131. INFLUENCE OF REPEATED LOW DOSE IRRADIATION WITH X-RAYS AND NEUTRONS ON MAMMARY GLAND CARCINOGENESIS IN THE RAT

Broerse, J. J., vanBekkum, D. W., Hollander, C. F., vanZwieten, M. J., Central Org. for Applied Scientific Research T N O, Radiobiological Inst. T N O, 151 Lange Kleiweg, P.O. Box 5815, 2280HV Rijswijk, Netherlands

Experiments on the influence of repeated low dose irradiation on mammary gland carcinogenesis are of great interest because of the following two reasons: 1. In order to understand the process of carcinogenesis it is important to investigate the relative contributions from repair of sublethal damage, repopulation and tissue turnover during the course of a fractionated irradiation with, for instance, 10 fractions every month. 2. In order to estimate the risks of routine diagnostic procedures such as mammography it will be important to obtain data from model studies in animals which could serve as a baseline for the human situation. In view of the possible synergistic effect of combinations of radiation and treatment with hormones the combined effect of fractionated radiation and estrogen treatment will also be investigated. It is expected that the influence of some of these factors will diminish considerably if radiation with a higher LET is employed. Consequently, it is recommended that parallel experiments with high LET radiations, preferably with neutrons of 0.5 MeV energy, are included in such a study.

In 1978 a total number of 900 rats have been exposed to different schemes of such fractionated irradiations with X-rays, gamma-rays and 0.5 MeV neutrons. The total project will take at least three years. The present application describes the program for the third year.

132. MYELOID LEUKEMIA IN IRRADIATED MICE - INDUCTION AND BIOLOGICAL CHARACTERISTICS

Mole, R. H., Meldrum, R. a., Davids, J. A., Medical Research Council, Radiobiology Unit, Harwell, Didcot, Oxfordshire OX11 0RQ, England, United Kingdom

OBJECTIVE: To provide an experimental model facilitating investigation of the development of myeloid leukemia in vivo and of the dose-response relationships of its induction.

APPROACH: Induction is by exposure to ionizing radiation (X- and gamma rays, fission neutrons) using a long-established genetically defined inbred mouse strain (to allow cell transfer experiments in vivo) with a low natural frequency of myeloid leukemia (to enhance the significance of low frequencies of induction).

PROGRESS: The dose response for brief exposure to X-rays was highly non-linear and initial experiments seemed to confirm the necessity of taking cell sterilization into account when interpreting the quantitative aspects of induction (*Nature*, 272, 455-6, 1978).

133. RELATIVE BIOLOGICAL EFFECTIVENESS (RBE) OF 50 MEV NEUTRONS COMPARED TO CO60 GAMMA RAYS IN INDUCING LEUKEMIAS AND CANCERS IN MICE

Maisin, J. R., Centre d'Etude de l'Energie Nucleaire, Dept. of Radiobiology, 200 Boerentang, B2400 Mol, Belgium

OBJECTIVES: To determine the relative biological effectiveness (RBE) of 50 MeV cyclotron produced neutrons compared to 60Co gamma rays in leukemia and cancer induction in mice.

APPROACH: Single dose of irradiation and irradiation given in 10 daily fractions will be compared in order to assess the number of non-reparable and reparable lesions resulting from the two radiation schedules. In addition, localized irradiation on the thorax and abdomen will be compared with total body irradiation. Two strains of mice, C57BL and BALB/c, were selected for this study based on the difference in their spontaneous leukemia and cancer incidences. In order to obtain dose-effect relationships, different groups of animals will be irradiated with increasing doses of gamma rays (25 to 600 rad) and neutrons (10 to 320 rad). Dead mice will be autopsied and the causes of death determined. In addition, groups of mice will be sacrificed at regular intervals and the number of the different types of cancer scored in order to detect an eventual acceleration in the induction of different types of tumors.

134. RADIATION CARCINOGENESIS

Warren, S., New England Deaconess Hospital, 185 Pilgrim Rd., Boston, Massachusetts, 02215, United States of America

This investigation of radiation and carcinogenesis is primarily concerned with the induction of tumors of various sorts in mice and rats and the relative responsiveness of the parent tissue. Much of the radiation is administered in the 1000 rad dose range using chiefly 250 kvp x-rays. Both leukemia and solid tumors are used as indicators of effect. Parabiosis is used in some animals to permit survival at high dose levels of radiation and also to produce hormonal imbalance. Hormonal imbalance appears to be an important factor in relation to the induction of mammary and adrenal cortical tumors among others. Asbestos is used as a cocarcinogen in some animals. Methylcholanthrene is used in combination with radiation and with asbestos. The asbestos is injected intratracheally, intrapleurally, and intraperitoneally in both rats and mice.

135. CARCINOGENIC EFFECTS OF SMALL DOSES OF X-RAYS GIVEN SHORTLY AFTER BIRTH

Hulse, E. V., Walding, P., Medical Research Council, Radiobiology Unit, Harwell, Didcot, Oxfordshire OX11 0RQ, England, United Kingdom

OBJECTIVE: To find whether mice irradiated shortly after birth develop an increased incidence of tumors.

APPROACH: Mice are exposed to 5-150 rad of X-rays either within 24 hrs. or 48 hrs. of birth.

136. PROBLEMS IN RADIATION EMBRYOLOGY

Brent, R. L., Jensh, R. P., Wheelock, E. F., Gorson, R. O., Schneiderman, M., Thomas Jefferson University, Jefferson Medical College, Dept. of Pediatrics, 1025 Walnut St., Philadelphia, Pennsylvania, 19107, United States of America

Our laboratory will continue its research on the effects of irradiation on mammalian embryonic development. These projects are as follows: 1. The Effect of Embryonic X-Irradiation on Leukemia Induction in Prenatally Exposed Mice (ongoing): These studies involve the exposure of strains of leukemia-sensitive and leukemia-resistant mice to prenatal radiation. The animals are studied for the postnatal occurrence of leukemia; 2. Determining the Threshold Dose or Low-Dose Incidence for Congenital Malformations in the Developing Rat Embryo (ongoing): Our studies involve much larger numbers of animals than have been utilized in previous investigations and detailed analysis of the internal and external morphology. The threshold studies proposed should provide important information on the frequency of malformations at low doses; 3. The Effect of X-Irradiating the Rat Embryo on the First Day of Gestation: Determination of those Factors or Circumstances which are Responsible for Embryonic Death; 4. The Effect of Neutron Radiation on Embryonic Development in the Rat; 5. The Effect of X-irradiation on Cell Kinetics of the Developing Rat Embryo during Organogenesis: Utilizing the techniques of the cell biologist,

the cell cycle parameters will be studied in irradiated and control embryos during the period of organogenesis. Dissociated early embryos will be studied for cell number, cell volume and cell viability. Autoradiographic techniques with tritiated thymidine will be used to describe the cell kinetics; 6. The Indirect effect of Embryo-Culture as a Method of Evaluating the Mechanism of Radiation Teratogenesis; 8. Irradiation of the Developing Yolk Sac: Specific antibodies against various components of the developing yolk sac will be labeled with ²¹¹At or ¹²⁵I in an effort to specifically irradiate the developing rat yolk sac. Because of the nature of radiation embryological effects, a great deal of information obtained in radiation embryological experiments can be prudently applied to the human situation.

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137. TRANSFORMATION IN VITRO WITH SPLIT DOSES OF RADIATION

Miller, R. C., Columbia University, College of Physicians & Surgeons, Dept. of Radiology, 630 W. 168th St., New York, New York, 10032, United States of America

In a practical situation, when radiation is received by radiation workers or members of the general public, the dose is delivered in a series of small dose-fractions or continuously at low dose-rate. By contrast, most carcinogenesis studies with experimental animals have involved single large doses of radiation.

Recently developed methods of in vitro oncogenic transformation are being used to investigate the effect of dose fractionation and variations in dose-rate, both for x-rays and for cyclotron produced neutrons. The C3H/10T1/2 cell line has been shown to be readily transformable with chemical carcinogens or ionizing radiations. The rate of transformations have been determined for single and split doses over a wide range of dose levels, both for x-rays and for neutrons. Experiments with x-rays indicate that at low dose levels below 100 rad, splitting the dose into two fractions separated by a time interval of 5 hours increases the incidence of transformed clones compared with the same total dose delivered in a single exposure. By contrast, at high doses, dose fractionation results in a decrease in the transformation rate. Dose splitting of neutrons into two fractions separated by five hours showed no change from a single dose in the range from 1 rad to 200 rad. Current research in this area is directed at using lower doses of neutrons. These findings, which have important implications in radiation protection and in the interpretation of risk estimates, will be confirmed and extended. In addition, the effect of continuous low dose-rate exposures will be investigated.

BIBLIOGRAPHIC REFERENCES: Miller, Richard C., Hall, Eric J., and Rossi, Harold H., Oncogenic Transformation of mammalian cells in vitro with split doses of x-rays. Proc. Natl. Acad. Sci. 76: No. 11, 5755-5758, November, 1979.

138. PRODUCTION OF ONCOGENIC PURINE N-OXIDES BY IONIZING RADIATION

Parham, J. C., Sloan Kettering Inst. for Cancer Research, 410 E. 68th St., New York, New York, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

139. MEDIUM (LOW) LEVEL LONG-TERM EFFECTS OF RADIATION

Ullrich, R. L., U.S. Dept. of Energy, Oak Ridge National Lab., Biology Div., P.O. Box X, Oak Ridge, Tennessee, 37830, United States of America

The purpose of this project is to examine the tumorigenic effects of low dose rate gamma ray exposures in several murine tissues which are sensitive to tumor induction with radiation and whose tumorigenic response to acute gamma rays is well defined. The experiments are comparing the effects of high (40 rad/min) and low (1 rad/day) dose rate gamma ray exposure on the induction of lung adenocarcinomas, mammary adenocarcinomas, and ovarian tumors in the BALB/c mouse. The lack of

adequate information on dose rate effects of low LET radiation remains a serious problem when the risks of environmental radiation exposures are assessed. This is true for the assessment of carcinogenic risks from exposure to low LET radiation and for the assessment of the relative biological effectiveness of high LET radiation. Since similar studies, examining neutron effects using these endpoints, are being conducted in our laboratory, this study will provide information on the low dose rate effects of gamma rays; it will also provide information necessary to evaluate the relative biological effectiveness of neutrons. In addition, such a comparison will provide new information on the relative degree of recovery from carcinogenic injury which can occur after gamma and neutron irradiation.

140. RADIOBIOLOGIC PARAMETERS OF RADIATION ONCOGENESIS (RATS)

Burns, F. J., New York University, School of Medicine, Dept. of Environmental Medicine, 550 1st Ave., New York, New York, 10016, United States of America

This is part of a broader project. A summary of this subproject is not available.

141. SURVIVAL AND CARCINOGENESIS IN TRANSPLANTED HEPATOCYTES

Jirle, R. L., Michalopoulos, G., Duke University, School of Medicine, Dept. of Radiology, Box 3711, Durham, North Carolina, 27710, United States of America

The primary objective of the proposed project is to quantify the probability of malignant transformation per surviving clonogenic cell per dose of carcinogen when hepatocytes are exposed to AAF, aflatoxin, and low LET radiation, and to determine whether this probability is cell cycle dependent. The project shall be divided into both short-term studies devoted to the development of a cell survival assay system and its application and long-term carcinogenesis experiments. The former will be accomplished by transplanting unexposed and carcinogen exposed monodispersed hepatocytes into the dorsal fascia of isogenic rats manipulated to provide an adequate stimulus for subsequent hepatocyte proliferation (e.g., partial hepatectomy). The chemical carcinogenesis studies entail in vitro exposure of enzymatically dispersed hepatocytes, transplantation of these cells into isogenic rats and subsequent observation for tumor development. Similar studies will be performed with hepatocytes exposed to low LET radiation while in various stages of their cell cycle. By the use of these methods we hope to: 1) develop a system whereby the number of cells which survive an exposure to a carcinogen can be quantitated, 2) minimize the abscopal effects of chemical carcinogens and ionizing radiation, 3) develop a system where the probability of malignant transformation of hepatocytes exposed either in vitro or in vivo to either chemical carcinogens or radiation can be determined as a function of surviving clonogenic cells rather than total number of initially exposed cells, and 4) to ascertain whether the probability of malignant transformation is dependent upon cell cycle stage.

142. ANALYSIS OF RESEARCH ON EFFECTS OF IONIZING RADIATION

Weiss, D. L., National Academy of Sciences, 2101 Constitution Ave. N.W., Washington, District of Columbia, 20037, United States of America

Conduct an analysis of the Federal Government's activities in ionizing radiation research, with emphasis on the human health and related biological aspects of that research, with respect to effects of ionizing radiation.

143. INHALATION HAZARD TO URANIUM MINERS

Cross, F. T., U.S. Dept. of Energy, Battelle Pacific Northwest Lab., P.O. Box 999, Richland, Washington, 99352, United States of America

The purpose of this project is to determine the specific biological effects of daily exposures to known levels of pathogenic uranium mine air contaminants, using both large and small experimental animal models of human respiratory disease.

144. INHALED TRANSURANICS IN RODENTS

Sanders, C. L., U.S. Dept. of Energy, Battelle Pacific Northwest Lab., P.O. Box 999, Richland, Washington, 99352, United States of America

Inhalation exposure constitutes the most likely route of entrance of transuranics into the body following release of transuranic compounds. Lung cancer is the most likely consequence of that exposure. The purpose of this project is to examine in small mammals, the fate, effects and pathogenesis in the lung and elsewhere following inhalation of transuranic compounds. This project should provide data required for hazard evaluation and establishment of permissible exposure limits to man for inhaled transuranics, particularly with respect to radiation dose and dose-distribution.

145. RADIATION DOSIMETRY AND CARCINOGENESIS

Albert, R. E., New York University, School of Medicine, Dept. of Environmental Medicine, 550 1st Ave., New York, New York, 10016, United States of America

This is part of a broader project. A summary of this subproject is not available.

146. LATE SOMATIC EFFECTS OF ENERGY POLLUTANTS

Walburg, H. E., U.S. Dept. of Agriculture, Comparative Animal Research Lab., 1299 Bethel Valley Rd., Oak Ridge, Tennessee, 37830, United States of America

Many toxic agents are carcinogenic or produce other late effects at levels which do not cause detectable acute injury. The principal current experiment is a study of irradiation effects in burros begun in 1951. The most interesting finding to date is that the incidence of spontaneous neoplasia in this species is very low and that exposure to ionizing radiation does not seem to be carcinogenic. Continuation of the study is needed to place these results on a statistically valid basis. Studies on platelet aggregation as influenced by age, species, and prior irradiation treatment are also being conducted. Preliminary experiments on the influence of species on chemical carcinogenesis have been initiated.

BIBLIOGRAPHIC REFERENCES: Schneider, M.D., F.H. Cross and J.N. Dumont, Burro Aortic Collagen: Platelet Aggregating Activity and Ultrastructural Changes Induced by Plasma, *Am. J. Vet. Res.* 39 (Mar 1978); Schneider, M.D., Platelet Affinity for Burro Aortic Collagen, *Am. J. Vet. Res.* 38: 1561-1570 (1977).

C. RADIONUCLIDE CARCINOGENICITY

1. CARCINOGENICITY OF ALPHA-EMITTING RADIONUCLIDES

147. INFLUENCE OF DISTRIBUTION OF RADIATION DOSE TO THE LUNG ON TUMOR INCIDENCE IN RATS

Batchelor, A. L., Ash, P., Gore, D. J., Jenner, T., Patrick, G., Medical Research Council, Radiobiology Unit, Harwell, Didcot, Oxfordshire OX11 0RQ, England, United Kingdom

OBJECTIVE: To investigate whether, or not, 'point' sources of radioactivity (so-called 'hot particles') within the lung are more carcinogenic than the same activity spread uniformly through the lung.

APPROACH: 'Hot particles' are created in the lung by exposing rats bearing inhaled particulate uranium dioxide to a fluence of approximately 10 to the 12th power neutrons for 2.5 minutes. Atoms of ²³⁵U in these oxide particles are fissioned during the neutron exposure and ejected fission fragments irradiate the surrounding tissue to simulate 'hot particles'.

PROGRESS: Rats exposed in the above fashion are entering the second year of the study and histological examination will commence early in 1980. A preliminary report is in press (Batchelor et al., *International Journal of Radiation Biology*, 1980).

148. EFFECT OF LET AND MICRODISTRIBUTION OF RADIATION ON THE INDUCTION OF TRANSFORMATION IN VITRO AND IN VIVO

Little, J. B., Harvard University, Boston Campus, School of Public Health, Dept. of Physiology, 55 Shattuck St., Boston, Massachusetts, 02115, United States of America

Slow growing cell cultures will be exposed to alpha irradiation from ²³⁸PuO₂ particles and uniform alpha radiation exposure. Their ability to transform will be related to exposure conditions. These data will be compared to tumor induction in

rat lung following particulate and more uniform exposure to alpha particles. The effects of non-uniform dose distribution should be determined and used for hazard evaluation.

149. TUMORIGENESIS IN THE LUNG

Thomas, R. G., U.S. Dept. of Energy, Los Alamos Scientific Lab., P.O. Box 1663, Los Alamos, New Mexico, 87544, United States of America

The objective is to evaluate the carcinogenic potential of various forms of radionuclides in the lung. Primary interest has been on the tumorigenicity of plutonium isotopes (238, 239) as sources of alpha particle radiation. Earlier research dealt with the lodging of ceramic particles containing radioactivity of various source strengths into Syrian hamster lungs, by injection via the jugular vein. This form of exposure has changed almost completely to the inhalation route, giving a diffuse irradiation of the lung, as opposed to the discrete hot particle situation after injection. It has become obvious that diffuse radiation to the lung is much more tumorigenic than when the same source strength is in discrete particles.

150. DOSE RESPONSE RELATIONSHIPS FOR INHALED PLUTONIUM RADIONUCLIDES

Muggenburg, B. A., Lovelace Center for the Health Sciences, Inhalation Toxicology Research Institute, 5200 Gibson Blvd. S.E., Albuquerque, New Mexico, 87108, United States of America

Studies are being conducted in beagle dogs and rodents to define relative importance of homogeneity vs non-homogeneity of alpha radiation dose to lung, importance of local dose around a particle, effect of particle size, and influence of age at exposure to inhaled plutonium aerosols. Dogs were exposed to monodisperse aerosols of either $^{239}\text{PuO}_2$ (aerodynamic diameters (AD) of 0.75, 1.5 or 3.0 micrometers) or $^{238}\text{PuO}_2$ (ADS of 1.5 or 3.0 micrometers) to achieve graded initial lung burdens. For a given ILB, average radiation dose to the lung will be constant but local dose rate around inhaled particles varies by a factor of about 3000 from the 0.75 micrometers AD $^{239}\text{PuO}_2$ particle to the 3.0 micrometers AD $^{238}\text{PuO}_2$. At early times after exposure, a few dogs died with radiation pneumonitis. Lung tumors and bone tumors are now occurring in some dogs exposed to $^{238}\text{PuO}_2$. Rodents are being utilized to supplement studies in dogs. Hamsters were exposed to polydisperse aerosols of $^{239}\text{PuO}_2$, $^{239}\text{Pu}(\text{NO}_3)_4$, and $^{238}\text{PuO}_2$. Pulmonary neoplasia was not observed in these animals but was observed in hamsters exposed by instillation of ^{210}Po on hematite particles. Because of these unexpected results, additional studies are being initiated in Fischer-344 rats.

151. MECHANISM OF INDUCTION OF OSTEOSARCOMA BY PLUTONIUM-239 IN MICE

Howells, G. R., Ash, P., Green, D., Medical Research Council, Radiobiology Unit, Harwell, Didcot, Oxfordshire OX11 0RQ, England, United Kingdom

OBJECTIVE: To study the distribution of ^{239}Pu in bone and to relate this to incidence and site of origin of osteosarcoma in exposed mice.

APPROACH: Mice are injected intravenously with ^{239}Pu and the site of deposition and subsequent redistribution is identified microscopically using neutron-induced autoradiography. The dose to cells on bone surface is subsequently calculated. Bones are also examined for early neoplastic (and non-neoplastic) changes by light and transmission electron microscopy.

PROGRESS: The techniques for identifying the location of ^{239}Pu in bone and for calculating the likely dose to cells on bone surface are now well established in this laboratory (D. Green et al., *Phys. Med. Biol.*, 22, 284-297 (1977); *Int. J. Radiat. Biol.*, 1, 27-36 (1978)). In the most recent study 1 microcurie of $^{239}\text{Pu/kg}$ body weight has been injected intravenously into mice and we are following these animals for an expected lifetime ending early 1980.

152. INVESTIGATION OF A POSSIBLE RELATIONSHIP BETWEEN ALPHA-EMITTING BONESEEKING RADIONUCLIDES AND THE INDUCTION OF MYELOID LEUKEMIA IN MICE

Humphreys, E. R., Loutit, J. F., Stones, V. A., Medical Research Council, Radiobiology Unit, Harwell, Didcot, Oxfordshire OX11 0RQ, England, United Kingdom

OBJECTIVE: To establish whether there is a regime of administration of alpha-emitting osteotrope which simulates the induction of myeloid leukemia by X-rays.

APPROACH: Protraction of administered activity is being explored as a means of maximizing any effect; the coincidental induction of osteosarcoma is being sought for comparison.

Complementary experiments will investigate the effects of similar alpha-osteotropes on hematopoietic stem cells by the measurement of colony-forming units in spleen and from ^{125}I -labelled cell kinetics.

153. MODELS AND MECHANISMS OF DOSE-RESPONSE

Marshall, J. H., U.S. Dept. of Energy, Argonne National Lab., 9700 S. Cass Ave., Argonne, Illinois, 60439, United States of America

The objective is to formulate a theory of the induction of bone cancer by alpha particle that synthesizes data from cell culture, animals and man within a logical, mechanistic framework that can be tested by experiment. Approaches will include in-vitro study of the properties of human cell tumors and the effects of radiation on cells in tissue culture; study of the viral and immune status of living radium patients; and computer modeling to fit postulates, mechanisms and data in a three-dimensional analysis of tumor rate, dose rate and time. Studies will result in a determination of host factors involved in cancers produced by irradiation; and dose-response curves, based on mechanisms, that fit data for man, animal, and cell cultures on induction of bone cancer by alpha-particle irradiation. These theoretical and mechanistic results are needed for assessment of risk at low levels from radionuclides in nuclear fuel cycles.

BIBLIOGRAPHIC REFERENCES: Marshall, J.H., and P.G. Groer, A Theory of the Induction of Bone Cancer by Alpha Radiation, *Radiation Research* 71(1): 149-192(Jul 1977).

154. ALPHA RADIOBIOLOGY

Morken, D. A., University of Rochester, Graduate School, Administration Bldg., Rochester, New York, 14627, United States of America

This research program provides information on the biological effects, and their mechanisms, of internal alpha irradiation. Its immediate purpose has been to inquire into the nature of the radiological effects of radon and its decay products, uncomplicated and uncompromised by the presence of other possible tumorigenic agents, to demonstrate whether alpha radioactivity alone could be the stimulus to produce lesions which would develop into lung cancer and to provide information on the biological mechanisms which may be involved in these various responses.

2. CARCINOGENICITY OF BETA-EMITTING RADIONUCLIDES

155. CARCINOGENIC AND GENETIC EFFECT OF LOW LEVEL TRITIUM

Mewissen, D. J., Rust, J. H., Ugarte, A. S., University of Chicago, Pritzker School of Medicine, Dept. of Radiology, 5801 S. Ellis Ave., Chicago, Illinois, 60637, United States of America

This research is primarily aimed at assessing comparatively the potential carcinogenic potency of tritiated water following continuous low level exposure of C57 Black/6 M mice from conception, birth or weaning for the rest of the lifespan, versus administration of tritiated thymidine, uridine or leucine at birth, in relation with specific patterns and sites of incorporation, as observed in our previous research. The completed carcinogenic studies will require observation of 4,363 mice during their entire lifespan. Microscopic pathology on all gross abnormalities and on most organs and tissues is aimed at providing an extensive definition of the basic tumor spectrum in controls and of the modulated spectra in experimental mice. Actuarial analysis based on age specific mortality and tumor incidence rates observed to date revealed: (1) an extremely high incidence of naturally occurring tumors in control mice and (2) a dose-dependent induction of adenocarcinomas of the ileum in mice exposed to tritiated thymidine at birth. In an attempt to evaluate the potential cumulative genetic injury of tritium, three sublines have been propagated from three sibling couples whose male parent was at weaning time, either injected with tritiated thymidine or exposed for 35 days to tritiated drinking water or

exposed to natural water. At the present time, 14 successive generations have been produced. It is planned to carry the experiment to the 20th generation. An increasing reduction of offspring through successive generations has been consistently observed in sublines exposed to tritium. A significant increase in dominant lethal mutation was observed in the unexposed F2 offspring from the 9th generation in sublines exposed to tritium. The F1 offspring is currently under observation for tumor incidence in an attempt to detect any possibly shift from the basic tumor spectrum. A duplicate assessment of reproductive fitness and dominant lethal mutations is planned at the 20th generation, using part of the F2 offspring. The F1 and the unused F2 offspring will be assigned to parallel long-term studies investigating any possible shift from the basic tumor spectrum.

156. TOXICOLOGY OF KRYPTON-85

Ballou, J. E., U.S. Dept. of Energy, Battelle Pacific Northwest Lab., P.O. Box 999, Richland, Washington, 99352, United States of America

This project is concerned with evaluating the biological effects of exposure to ^{85}Kr in rodents and larger animal species. Included are both short-term and chronic exposure studies to determine tissue distribution and retention kinetics for metabolic modeling purposes, as well as long-term biological effects, for purposes of defining the tissues at risk. Also included are dose-effect studies to determine tumorigenicity, teratologic potency, and capacity to induce changes in lung biochemistry, pulmonary function, and hematopoiesis.

BIBLIOGRAPHIC REFERENCES: Williard, D.H. and J.E. Ballou, The Disposition of ^{85}Kr in the Rat, *Health Phys.* 33: No. 6, 1978 (abstract).

157. HEALTH EFFECTS - KRYPTON

Kirk, W. P., U.S. Environmental Protection Agency, Health Effects Research Lab., Research Triangle Park, North Carolina, 27711, United States of America

To define the biological hazard from Kr-85 released to the environment, experimental animals are being exposed to a cloud of Kr-85 . The study will evaluate the long-term effects of acute exposure in guinea pigs and acute and chronic exposure of rats. The distribution, rate of removal, the dose to several organs, and the acute LD/sub 50-30/ for inhalation exposure were determined in guinea pigs. Following this evaluation, rats, Chinese hamsters and guinea pigs were exposed in beta-infinite geometry to determine the acute LD/sub 50-90/. Life time observation to evaluate long term hazards is in progress on guinea pigs surviving the LD/sub 50/ study and rats exposed in a skin-tumor induction study. Rats will be exposed in utero and chronically postnatally to Kr-85 in beta infinite geometry at multiples of the occupational MPC/sub a/. Preliminary data indicate an excess of lymphocytic leukemias in female guinea pigs.

158. DOSE-RESPONSE RELATIONSHIPS FOR INHALED BETA-EMITTING RADIONUCLIDES

Hahn, F. F., Lovelace Center for the Health Sciences, 5200 Gibson Blvd. S.E., Albuquerque, New Mexico, 87108, United States of America

This project is directed to establishing dose-effect relationships for inhaled beta-emitting radionuclides. Animals have been exposed by inhalation to selected beta-emitting radionuclides with different physical parameters which result in different radiation dose patterns in the body. Animals are maintained for their life span to determine longevity and the types of diseases induced. Beagle dogs have been exposed once to graded levels of relatively soluble ($^{90}\text{Sr-Cl}_2$, $^{144}\text{Ce-Cl}_3$, $^{91}\text{Y-Cl}_3$, $^{137}\text{Cs-Cl}$) and relatively insoluble forms (^{90}Y , ^{91}Y , ^{144}Ce and ^{90}Sr in fused aluminosilicate particles). Initial body burdens range from very high, which cause acute injury and early deaths, to low, which are equivalent to the maximal permissible body burden for man. Dogs are exposed at 13 months of age except for one study of influence of age at exposure on biologic effects in which dogs 3 months or 8 to 10.5 years old are exposed to ^{144}Ce in fused aluminosilicate particles. Syrian hamsters, C57BL/6J mice and BALB/c mice have been exposed to aerosols of $^{144}\text{Ce-O}_2$ to determine the differences in species response to inhaled beta-emitting radionuclides.

BIBLIOGRAPHIC REFERENCES: Benjamin, S.A., F.F. Hahn and B.B. Boecker, Effects of Chronic Pulmonary Irradia-

tion on Peripheral Lymphocytes and Their Function in the Dog, *Radiat. Res.* 75: 121-137 (1978).

159. TOXICITY STUDIES OF INHALED BETA-EMITTING RADIONUCLIDES

Hahn, F. F., McClellan, R. O., Boecker, B. B., Lovelace Inhalation Toxicology Research Inst., P.O. Box 5890, Albuquerque, New Mexico, 87115, United States of America

An experimental approach to evaluating the toxicity of inhaled beta-emitting relatively soluble radionuclides such as $^{90}\text{SrCl}_2$, $^{144}\text{CeCl}_2$, $^{91}\text{YCl}_3$, and $^{137}\text{CsCl}$ in laboratory animals is being developed. Initial lung or whole-body radionuclide burdens were selected for early deaths caused by severe lesions at the highest exposure levels, and for more subtle changes such as neoplasia at lower exposure levels. For radionuclides inhaled in relatively soluble forms, long-term effects have been seen in the liver, skeleton, respiratory tract, and other tissues. Relatively insoluble forms are mainly associated with the lung and contiguous tissues. In all experiments, emphasis is placed on evaluating the influence of radiation dose rate and total dose on the resulting dose-response relationship. These studies with young adult dogs are complemented with comparable studies in other species (mice, rats, and Syrian hamsters) and with animals of different ages (immature, aged). This basic approach, with emphasis on factors that alter the resulting radiation dose pattern, offers the maximum likelihood of meeting the continuing, and not always predictable, needs for information on the toxicity of inhaled beta-emitting radionuclides that may be encountered in nuclear industry operations.

The radionuclides being studied, all important fission products, are present in substantial quantities in many nuclear processes and thus are potential airborne pollutants. The radionuclides represent a range of physical and half-lives which provide an opportunity to assess the influence of radiation dose rate and total radiation dose on the dose-response relationships for inhaled radionuclides. Since all of these radionuclides and their associated daughters are energetic beta emitters and a large number of particles are deposited in the lung, there is nearly uniform beta irradiation of the lung. All of the aerosols have been distributed lognormally with geometric standard deviations greater than 1.2.

160. TOXICITY OF INHALED STRONTIUM-90 DICHLORIDE IN DOGS

Muggenburg, B. A., Hahn, F. F., Boecker, B. B., Lovelace Inhalation Toxicology Research Inst., P.O. Box 5890, Albuquerque, New Mexico, 87115, United States of America

The metabolism, dosimetry, and biological effects of inhaled $^{90}\text{SrCl}_2$ in the Beagle dog are being studied to provide a basis for assessing the consequences of inhaling ^{90}Sr such as might be released in certain nuclear accidents. Seventy-two dogs were exposed to aerosols containing ^{90}Sr resulting in initial body burdens ranging from 2.5 to 250 microcuries $^{90}\text{Sr}/\text{kg}$ body weight. Forty-eight of these dogs are being maintained for lifetime observation along with 25 unexposed dogs. The long-term retained burden (LTRB) in these dogs ranged from 1 to 120 microcuries $^{90}\text{Sr}/\text{kg}$. The other 24 dogs, with a mean LTRB of 38 microcuries $^{90}\text{Sr}/\text{kg}$, were assigned to a sacrifice study. Nine of these dogs were sacrificed up to 1 year after inhalation of ^{90}Sr . To date, 60 ^{90}Sr -exposed dogs have died or have been euthanized, six during the first 31 days after inhalation of ^{90}Sr with bone marrow aplasia and 54 between 585 and 5109 days after inhalation of ^{90}Sr . The latter group includes 32 dogs with bone-related neoplasms, two with upper respiratory tract carcinomas, and five dogs with various diseases of the lower respiratory tract and heart. The other 15 dogs had diseases in organs that received little or no radiation dose such as the gastrointestinal tract, urinary tract, and the central nervous system. The skeletons of the dogs dying with bone-related neoplasms received initial radiation dose rates of 3.2 to 55 rads/day and cumulative doses to death of 2800 to 22,000 rads. Fourteen control dogs have died or been euthanized, two during the last year with mammary carcinoma and intestinal lymphosarcoma. Serial observations are continuing on the six surviving ^{90}Sr dogs and six controls.

Strontium-90 is one of the fission product radionuclides that predominates in a nuclear reactor inventory after a period of sustained operation. It was selected for study because of its

high probability for release in certain types of reactor accidents as well as its long half-life, sustained dose rate, and energetic beta emissions.

161. TOXICITY OF YTTRIUM-90 IN A RELATIVELY INSOLUBLE FORM BY DOGS

Merickel, B. S., Hobbs, C. H., Hahn, F. F., Lovelace Inhalation Toxicology Research Inst., P.O. Box 5890, Albuquerque, New Mexico, 87115, United States of America

Studies on the metabolism, dosimetry, and biological effects of inhaled ^{90}Y in fused aluminosilicate particles in the Beagle dog are in the process to assess the consequences of inhaling an energetic beta-emitting radionuclide that has a short effective half-life in the lung. A life span study is currently in progress in which 89 dogs were exposed to initial lung burdens ranging from 80 to 5200 microcuries/kg body weight, and 12 control dogs were exposed to stable yttrium in fused aluminosilicate particles. The doses achieved, short half-life, and relative insolubility of the ^{90}Y in this vector resulted in a radiation insult to the lung and surrounding structures at a relatively high, but rapidly decreasing, dose rate. To date, 46 exposed dogs have died: of these, 38 were from radiation pneumonitis; four were from primary pulmonary neoplasms; and four were from other causes. Forty-three surviving exposed dogs and 12 control dogs are being studied for the remainder of their life span.

This study is one of a series designed to determine the relationship of total radiation dose and radiation dose rate from inhaled beta-emitting radionuclides to observed biological effects.

162. TOXICITY OF INHALED YTTRIUM-90 TRICHLORIDE IN DOGS

Muggenburg, B. A., Hahn, F. F., Boecker, B. B., Lovelace Inhalation Toxicology Research Inst., P.O. Box 5890, Albuquerque, New Mexico, 87115, United States of America

As yttrium-91 is an important contributor to the total fission product radionuclide inventory of a reactor after periods of sustained operation, the metabolism dosimetry, and effects of inhaled $^{91}\text{YCl}_3$ in Beagle dogs are being studied to aid in assessing the biological consequences of nuclear accidents in which ^{91}Y or other radionuclides that produce a similar radiation dose pattern may be released. Forty-two dogs with ^{91}Y initial body burdens from 14 to 1300 microcuries/kg body weight were placed in four groups with mean lung burdens of 310, 180, 75, and 40 microcuries/kg body weight. These dogs and 12 control dogs are being maintained for lifetime observation. An additional group of four dogs with a mean initial ^{91}Y body burden of 180 microcuries/kg body weight were placed in a sacrifice study. Thirty-three of the exposed dogs and four of the control dogs have died. Eleven dogs within the highest activity level groups died or were euthanized at 12 to 33 days after inhalation of ^{91}Y with changes related to severe bone marrow damage and associated pancytopenia. Three dogs died with neoplasms of the upper respiratory tract and three dogs died with tumors of the lower respiratory tract from 2012 to 4563 days after exposure. One dog died with a liver related tumor. The remaining 15 dogs died from causes that appear not to be related to the radiation from ^{91}Y . Control dogs died of miscellaneous neoplastic and chronic diseases. Serial observations are continuing on 13 surviving exposed dogs and six surviving unexposed dogs.

163. TOXICITY OF YTTRIUM-91 INHALED IN A RELATIVELY INSOLUBLE FORM BY DOGS

Muggenburg, B. A., Hobbs, C. H., Hahn, F. F., Lovelace Inhalation Toxicology Research Inst., P.O. Box 5890, Albuquerque, New Mexico, 87115, United States of America

The radiation hazards from inhalation of ^{91}Y in fused aluminosilicate particles are being studied in Beagle dogs to assess the biological consequences of inhaling a relatively insoluble, energetic beta emitter with an intermediate effective half-life in the lung. A radiation dose pattern study in which 30 dogs were serially sacrificed from 0 to 320 days after inhalation exposure to ^{91}Y in fused aluminosilicate particles has been completed. A life span study is in progress in which 96 dogs were exposed to achieve initial lung burdens ranging from 11 to 300 microcuries ^{91}Y in fused aluminosilicate particles/Kg body weight, and 12 dogs were exposed to stable yttrium in fused aluminosilicate particles. To date, 60 dogs have died in the

longevity study between 113 and 3390 days after exposure. Forty of these dogs, with cumulative radiation doses to lung between 8300 and 60,000 rads, died with clinicopathologic findings of radiation pneumonitis and/or pulmonary fibrosis at 113 to 1011 days after exposure. Fifteen dogs that died between 1115 and 2841 days after exposure, with cumulative doses to lung ranging from 16,000 to 25,000 rads, had pulmonary carcinomas. Two dogs have died with hemangiosarcomas with primary sites not in the lung. Deaths are beginning to occur that cannot be easily associated with the radiation exposure. Thirty-six exposed dogs with doses to lung of 2400 to 18,000 rads and 11 control dogs remain alive and will be studied throughout the remainder of their life span.

164. SKIN TUMORS IN MICE AFTER EXTERNAL BETA-IRRADIATION

Hulse, E. V., Medical Research Council, Radiobiology Unit, Harwell, Didcot, Oxfordshire OX11 0RQ, England, United Kingdom

OBJECTIVE: To study the incidences and dose-response curves for skin tumors after a wide range of single doses given at 4 different dose rates; to study any alterations in the incidences of skin tumors when doses are divided into multiple equal fractions or two unequal fractions.

APPROACH: The work is an extension of previous studies using open-ended cylinders of ^{204}Tl as beta-sources (Br. J. Cancer, 21, 531 (1967); 23, 452 (1969); Int. J. Radiat. Biol., 14, 437 (1968). The non-neoplastic responses vary from slight depigmentation to moist desquamation and permanent epilation. The mice, both irradiated and unirradiated controls, are kept for their natural life-span as many tumors occur during the third year of life.

165. TOXICITY OF INJECTED CESIUM-137 CHLORIDE IN DOGS

Merickel, B. S., Hahn, F. F., Boecker, B. B., Lovelace Inhalation Toxicology Research Inst., P.O. Box 5890, Albuquerque, New Mexico, 87115, United States of America

Cesium-137, which in this text refers to Cs^{137} in equilibrium with its daughter Ba^{137} , has been of interest in this laboratory because of its uniform distribution in body tissues regardless of the mode of entry.

Studies of the metabolism, dosimetry, and effects of intravenously administered $^{137}\text{CsCl}$ in the Beagle dogs are being conducted to aid in assessing the biologic consequences of exposure to Cs^{137} that might occur in the event of certain nuclear accidents. Effects of the chronic, relatively uniform whole-body exposure produced by Cs^{137} are being compared with other diverse radiation dose patterns resulting from inhalation of radioactive aerosols. Fifty-four dogs that were injected with $^{137}\text{CsCl}$ and twelve control dogs are being observed for their life spans. Twenty-five of the dogs injected with $^{137}\text{CsCl}$ have died: eleven of hematologic dyscrasias, seven of neoplastic diseases, and seven from other diseases. Five control dogs have died: one from a neoplastic disease, and four from other diseases. Serial observations are continuing on the surviving twenty-nine $^{137}\text{CsCl}$ injected dogs and seven control dogs.

166. EFFECTS OF CHRONIC EXPOSURE TO INHALED RADIONUCLIDES

Lundgren, D. L., Lovelace Center for the Health Sciences, 5200 Gibson Blvd. S.E., Albuquerque, New Mexico, 87108, United States of America

Studies of the effect of repeated inhalation exposure, also designed to simulate chronic inhalation exposures, are in progress to assess effects on radiation dose pattern and the resulting biological effects. An apparent increase in the long-term retention of ^{137}Cs labeled fused aluminosilicate particles has been observed in beagle dogs exposed weekly for up to 40 weeks compared with dogs exposed once. Beagle dogs, mice and Syrian hamsters have been repeatedly exposed to ^{144}Ce in relatively insoluble forms. The only biological effect observed in the dogs to date (about 3 years) has been a lymphopenia. Significant increases in the incidence of pulmonary tumors and decrease in life span have been observed in mice and Syrian hamsters repeatedly exposed to $^{144}\text{CeO}_2$ compared with those exposed once. Syrian hamsters appear to be more resistant to the biological effects of repeated exposure than mice. Similar studies are in progress in SPF rats. Mice and Syrian hamsters

have been repeatedly exposed to inhalation to $^{239}\text{PuO}_2$ resulting in significantly shortened survival.

167. TOXICITY OF INHALED CERIUM-144 TRICHLORIDE IN DOGS

Merickel, B. S., Hahn, F. F., Boecker, B. B., Lovelace Inhalation Toxicology Research Inst., P.O. Box 5890, Albuquerque, New Mexico, 87115, United States of America

Cerium-144 which refers to Ce144 in equilibrium with Pr144 , is one of the fission product radionuclides that predominates in a reactor inventory after a period of sustained reactor operation.

The metabolism, dosimetry, and effects of inhaled $^{144}\text{CeCl}_3$ in Beagle dogs are being studied to assess the biological consequences of inhaling Ce144 that might be released in certain nuclear accidents. Studies have shown that the Ce144 deposited in the lung as $^{144}\text{CeCl}_3$ is translocated at a moderately rapid rate to the liver and the skeleton, and that significant radiation doses are accumulated by all three organs. Fifty-five dogs that inhaled $^{144}\text{CeCl}_3$ and fifteen control dogs are being observed for their life span. The Ce144 exposed dogs had long-term retained burdens that ranged from 2.6 to 360 microcuries Ce144/kg body weight. Of the fifty dogs that died after exposure to $^{144}\text{CeCl}_3$, fifteen died from early effects of radiation exposure, 26 from neoplastic diseases, and nine from other diseases. Seven controls have died; three were from neoplastic diseases, and four from other causes. Serial observations are continuing on the five surviving exposed dogs and eight control dogs.

168. TOXICITY OF CERIUM-144 INHALED IN A RELATIVELY INSOLUBLE FORM BY IMMATURE DOGS

Boecker, B. B., Hahn, F. F., Merickel, B. S., Lovelace Inhalation Toxicology Research Inst., P.O. Box 5890, Albuquerque, New Mexico, 87115, United States of America

The influence of age at exposure on the resulting patterns of deposition, retention, dosimetry, and biological effects from a single inhalation exposure to a relatively insoluble form of a beta-emitting radionuclide with a relatively long physical half-life is being investigated. Immature Beagle dogs (3 months of age) have been exposed once, by inhalation, to an aerosol of Ce144 incorporated in fused aluminosilicate particles. Eighteen of these dogs were serially sacrificed to study the patterns of deposition, retention, and dosimetry, and the remaining 49 dogs received graded initial lung burdens that ranged from 0.004 to 140 microcuries Ce144/kg body weight. These dogs are being observed over their life span for study of the resulting long-term biological effects. Five control dogs are also included in this study. To date, 13 of the Ce144 -exposed dogs in the longevity study and none of the controls have died. Dogs with the highest initial lung burdens of Ce144 died first (during the first 4 months) with radiation pneumonitis, pulmonary fibrosis, and congestive heart failure. Pulmonary hemangiosarcoma was the primary finding in dogs that died at 1.5 to 2 years after exposure. Deaths beyond that time have primarily involved extrapulmonary hemangiosarcomas. One dog, 627B, with an initial lung burden of 24 microcuries Ce144/kg body weight died during the past year at 2341 days after with a widely disseminated hemangiosarcoma showing heavy involvement of the liver and skin. Observations are continuing on the surviving 36 Ce144 -exposed and five control dogs.

It is important that age-related effects on the behavior and long-term biological effects of inhaled radionuclides be examined because a population of people that might be exposed in a nuclear incident could contain individuals differing widely in age. Three studies are in progress to examine possible age-related differences.

169. TOXICITY OF CERIUM-144 INHALED IN A RELATIVELY INSOLUBLE FORM BY AGED DOGS

Hahn, F. F., Boecker, B. B., Muggenburg, B. A., Lovelace Inhalation Toxicology Research Inst., P.O. Box 5890, Albuquerque, New Mexico, 87115, United States of America

The toxicity of relatively insoluble Ce144 inhaled by 8- to 10.5-year-old dogs is being investigated to provide information on age-related differences in the response of dogs to lung burdens of this fission product. These data on aged dogs will be compared to the results of similar studies of dogs exposed at

approximately 3 months or 12 to 14 months of age. Forty-two dogs were exposed, nose only, to aerosols of Ce144 in fused aluminosilicate particles to yield initial lung burdens of 2.2 to 75 microcuries/kg body weight, and 12 control dogs were exposed to nonradioactive fused aluminosilicate particles. To date, 37 dogs have died or were euthanized 197 to 2375 days after inhalation of Ce144 . The prominent findings were radiation pneumonitis in 19 dogs that died at early times with cumulative doses to lung of 20,000 to 74,000 rads, and neoplastic disease in six of 14 dogs that died 943 days after exposure or later. Pulmonary tumors were found in four of these dogs. However, only one of these tumors killed the dog. No hemangiosarcomas have been observed in this study. This result is in contrast to the results with immature or young adult dogs exposed to Ce144 . The difference may be a dose-related phenomenon since dogs which developed hemangiosarcomas had greater initial lung burdens of Ce144 . Aged dogs with similar burdens died at earlier times with radiation pneumonitis. Observations are continuing on the five surviving Ce144 -exposed and four control dogs.

The long-term dose-response studies in this laboratory are being conducted to provide information for predicting dose-response relationships in man. Most of these studies have involved young adult animals, the individuals in a population who are the most physiologically fit. Thus, dose-response predictions for man based on data obtained from exposure of young adults may underestimate the risk of exposure for older members of the population who are generally in poorer condition.

170. BIOLOGICAL EFFECTS OF REPEATED INHALATION EXPOSURE OF DOGS TO RELATIVELY INSOLUBLE AEROSOLS OF CERIUM-144

Boecker, B. B., Hahn, F. F., Muggenburg, B. A., Lovelace Inhalation Toxicology Research Inst., P.O. Box 5890, Albuquerque, New Mexico, 87115, United States of America

Beagle dogs were exposed repeatedly to a relatively insoluble form of Ce144 in fused aluminosilicate particles to study the deposition, retention, and long-term biological effects for comparison with data from dogs that were exposed only once to a similar aerosol. Four groups of nine dogs each were exposed once every 8 weeks for 2 years, 13 exposures, to achieve specified exposure goals. These goals were to increase the lung burden by 2.5 microcuries Ce144/kg body weight with each exposure to reestablish lung burdens of 9 or 4.5 microcuries Ce144/kg body weight, and to expose controls to fused aluminosilicate particles containing nonradioactive cerium. With these exposure sequences, the Ce144 -exposed dogs received increasing or relatively constant beta radiation dose rates in contrast to the steadily decreasing dose rate seen after a single inhalation exposure. Following completion of the exposure series, the dogs are being observed for the development of long-term biological effects. To date, nine exposed dogs and two controls have died or were euthanized. Although pulmonary hemangiosarcomas were a prominent finding in dogs exposed once to the same aerosol at a level that led to cumulative doses to lung similar to these repeatedly exposed dogs, only one has been observed in the repeatedly exposed dogs. Other effects of note to date include three pulmonary carcinomas, two hemangiosarcomas of the tracheobronchial lymph nodes, and one splenic hemangiosarcoma. Observations are continuing on the surviving 18 exposed and seven control dogs.

171. LIFETIME EFFECTS OF I-129

Book, S. A., University of California, Davis Campus, School of Veterinary Medicine, Dept. of Radiological Sciences, Davis, California, 95616, United States of America

Iodine-129, with a physical half-life of 16 million years, must be considered to be important in the long-term processing, handling, storage and disposal of spent fuel rods from light-water fission reactors. As an isotope of iodine it is readily incorporated into biological systems, and may, like the relatively short-lived ^{131}I , reach human populations by the atmosphere-vegetation-cow-milk-man pathway. Because of its long half-life, however, it may also pass through other pathways uncommon to ^{131}I , such as soil-vegetation-man, and vegetation-meat-man. Little information exists on the metabolism and effects of ^{129}I . Therefore, we have proposed to investigate ^{129}I in mammals, studying its metabolism by its target organ, the thyroid gland,

and the effects from its chronic, low-level ingestion. The effects of exposure to ¹²⁹I, as demonstrated by changes in thyroid function, life shortening, and tumorigenesis, require definition in order to adequately predict the actual hazards to human health resulting from processes at the end of the fission fuel cycle. The life time exposure of rats to high levels of dietary ¹²⁹I, as utilized in this study, will provide this requisite information.

172. COMPARATIVE RAT THYROID CARCINOGENESIS FROM IODINE-131 AND EXTERNAL RADIATION

Lee, W., Shleien, B., U.S. Dept. of Health & Human Services, Public Health Service, Food & Drug Admin., Bureau of Radiological Health, 5600 Fishers Ln., Rockville, Maryland, 20857, United States of America

The purpose of this study is to evaluate the absolute and relative risks of thyroid carcinoma incidence in rats within the dose range of 0 to 1000 rad from both internal I-131 and x-irradiations. Also, the possible synergistic effects between the thyroid and the pituitary gland will be investigated when either one or both organs are x-irradiated. Three thousand six-week-old Long Evans female rats were divided into ten treatment groups. Four groups were irradiated with localized x-ray to the thyroids at approximately the same doses as those in the I-131 irradiated groups; and two groups were irradiated for 440 rad with localized x-ray to either the pituitary alone, or both the pituitary and the thyroid. Animals will be kept for a period of 24 months after the irradiations. Pathology of the thyroid and the pituitary will be examined under double-blinded conditions. Only those animals which survive at least six months after the irradiation will be counted for statistical analysis. As of December 1979, all animals have been sacrificed and histological slides prepared, initial pathological evaluation of thyroids and pituitary tissue has commenced, and comparative pathology study and analysis are in progress.

173. ETIOLOGY OF HYPERPARATHYROIDISM - RADIATION EFFECTS

Paloyan, E., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, 5th Ave. & Roosevelt Rd., Hines, Illinois, 60141, United States of America

A high incidence of exposure to radiation in infancy, childhood, adolescence and young adulthood has been reported in patients with hyperparathyroidism proven by operation. Incidental observations of parathyroid tumors and even metastasizing cancer have been reported in rats exposed to either ¹³¹Iodine or external radiation during the course of studies of the effects of radiation on the development of thyroid tumors.

According to preliminary reports, in various stages of publication, the incidence of hyperparathyroidism in the radiation exposed population may be as high as 5 percent: although this is strong presumptive evidence for an etiologic relationship it certainly is not conclusive of a direct effect of radiation on the parathyroid glands and development of parathyroid hyperplasia and neoplasia. Furthermore, a controlled study of the effects of radiation on the development of parathyroid hyperplasia and tumors in experimental animals has not been reported.

We propose to test the hypothesis that radiation is an etiologic factor in the development of parathyroid tumors and hyperparathyroidism in rats. The rat was chosen as a suitable experimental model, since it has been used extensively in studies of calcium metabolism and the development of radiation associated thyroid tumors. This research is in progress. More data should be forthcoming within 24 months.

D. RADIATION COCARCINOGENESIS

1. COCARCINOGENICITY OF RADIATION AND CHEMICALS

174. CARCINOGENIC INTERACTIONS OF RADIATION AND CHEMICALS

Ullrich, R. L., Storer, J. B., Girardi, A. J., Oak Ridge National Lab., Radiation Carcinogenesis Unit, P.O. Box Y, Oak Ridge, Tennessee, 37830, United States of America

The objective of this project is to examine the nature of the interactions between radiation and chemicals at low doses and dose rates with particular emphasis on the potential tumor enhancing or cocarcinogenic activity of low dose rate radiation

exposures. Specifically this research will examine: 1) whether the carcinogenic effectiveness of the radiation dose influences of a chemical carcinogen and low dose rate radiation; 2) the influence of radiation quality on these interactions. In these studies the effects of varying doses and dose rates of ¹³⁷Cs gamma ray of fission neutron irradiation on the development of mammary adenocarcinomas in BALB/c mice after an initial exposure to low doses of 1,12-dimethylbenz(a)anthracene will be determined.

175. ENHANCED ORAL CARCINOGENESIS BY LOW DOSE X-RADIATION

Lurie, A. G., University of Connecticut, Health Center, School of Dental Medicine, Dept. of Oral Diagnosis, Farmington Ave., Farmington, Connecticut, 06032, United States of America

The tissues of the human oral cavity are frequently exposed to repeated low doses of known and suspected carcinogens such as tobacco and x-radiation from diagnostic medical and dental sources. Prior studies in our laboratory have shown synergy between concurrent applications of DMBA and exposures to modest levels of x-radiation in hamster cheek pouch tumorigenesis. This synergy was manifested by greater tumor incidence, shorter latent period, and greater tumor volume in animals receiving combined treatments than in animals receiving either treatment alone. We have also shown that low-level x-radiation causes a significant late increase in pouch vascular volume and that there is a significant interaction between the effects of x-radiation and DMBA or DMBA-induced tumors on pouch functional vasculature. The proposed studies would examine the effects of decreasing radiation dose on DMBA-induced tumor incidence and progression as well as the possible mechanistic role of radiation-induced hemodynamic changes in the induction and progression of these tumors. Young adult male Syrian golden hamsters will receive various doses of DMBA to the cheek pouch accompanied by repeated exposures of 1-20R x-radiation at weekly intervals. The temporal relationships between applications of chemical and radiation will vary. Animals will be observed for varying lengths of time; tumor latency, volume, incidence, histopathology and hemodynamics will be studied using observational, histopathological and radioisotopic techniques. It is anticipated that the results of these studies will clarify dose-response relationships and time-dose relationships of low level x-radiation in this system, leading to a more quantitative basis for calculations of human radiation risk estimates. Additionally, the data should provide information on the nature of radiation induced hemodynamic changes in the induction of epithelial neoplasms by low level exposures to multiple environmental carcinogens.

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176. TUMORIGENIC ACTION OF BETA, PROTON, ALPHA AND ELECTRON RADIATION ON THE RAT SKIN

Burns, F. J., New York University, School of Medicine, Dept. of Environmental Medicine, 550 1st Ave., New York, New York, 10016, United States of America

The investigation of the effect of ultraviolet light, carcinogenic chemicals, fractionation and age on the oncogenic response of rat skin to various types, doses, and dose rates of ionizing radiation is continuing. The existence of a linear term in the dose response relationship at low doses will be studied by means of a multiple dose fractionation schedule. Epidermal stem cell lethality following irradiation will be determined as a function of cell cycle and the kinetics of recovery for tumor induction and cell lethality in vivo will be compared. The carcinogenic interaction of the polycyclic hydrocarbon, DMBA, and electron radiation will be studied. The dependency of tumor induction on radiation dose rate will continue to be studied with emphasis on the proposed theoretical model. The importance of epidermal cells will be determined with respect to tumor induction. The kinetics of damage and repair of DNA in the epider-

mal cells following irradiation will be investigated and compared with cell lethality and tumor induction. Tumor yield in rat skin is roughly proportional to the square of the radiation dose for electrons and protons. The recovery half-time for tumor induction was about 4 hrs for electrons and substantial recovery was evident for protons. A model describing dose rate effects in tumor induction has been developed.

BIBLIOGRAPHIC REFERENCES: Burns, F.J., and R.E. Albert, The Additivity of Multiple Doses of a Liver Carcinogen in Rats, *Proceedings of Oak Ridge Associated Universities Workshop*, in press, 1978; Burns, F.J., et al Rat Skin Tumor Incidence Following Single and Fractionated Exposures to Proton Radiation, *Radiat. Res.* 74 (1978).

177. X-RAY MODIFICATION OF ETHYLNITROSOUREA TRANSPLACENTAL CARCINOGENESIS

Kalter, H., Warkany, J., Childrens Hospital, 561 S. 17th St., Livingston Pike, Columbus, Ohio, 43205, United States of America

Pregnant Sprague-Dawley rats were X-irradiated on the 16th day of gestation with 5-250 rad and injected intraperitoneally 4 days later with 10 mg/kg ethylnitrosourea (ENU). The offspring were observed over their life span for the appearance of neurogenic tumors. The frequency of animals that developed tumors surviving beyond 4 weeks of age was inversely related to X-ray dosage: a) about 15% after exposure to the largest doses; b) 39-46% after the intermediate ones, and c) 58-65% after the smallest. After treatment with ENU alone, 69% occurred. The reductions in tumor frequency were not due to increased mortality rate of tumor-prone animals either before or after the onset of tumor appearance. Mean offspring weight at 4 weeks and 4 months of age was inversely related to X-ray dosage but the weight of those animals developing tumors later was not significantly different from that of animals remaining tumor free. Mean time of tumor appearance and mean number of tumors per affected animal were unrelated to tumor frequency. Destruction of target cells does not sufficiently explain the results.

178. EFFECT OF X-IRRADIATION ON THE NEUROCARCINOGENIC EFFECT OF ETHYLNITROSOUREA (ENU)

Knowles, J. F., Medical Research Council, Radiobiology Unit, Harwell, Didcot, Oxfordshire OX11 0RQ, England, United Kingdom

OBJECTIVE: To investigate whether X-irradiation has a synergistic or an inhibitory effect on tumors of the nervous system produced by ENU.

APPROACH: Newborn rats are injected with ENU during the first 24 hours of life and during the second 24 hours of life are irradiated.

179. EFFECT OF URETHANE ON 32P-INDUCED LEUKEMIAS AND LYMPHOMAS IN MICE

Olinici, C. D., Dobay, O., Oncological Inst. of Cluj-Napoca, Laboratory of Cytogenetics, Republicii Nr. 34-36, R3400 Cluj-Napoca, Romania

OBJECTIVE: The influence of urethane on the incidence, histological type and chromosome constitution of the lymphoid malignancies induced by 32P in mice.

APPROACH: Two groups of mice were injected with 32P. One of them was treated with urethane. We are currently studying the latent period, extension and cytohistological characteristics of the neoplasias and we are trying to use a banding technique in order to see if there are chromosomal differences between these two groups.

180. CELLULAR AND MOLECULAR EFFECTS OF PSORALEN PLUS ULTRAVIOLET LIGHT

Kraemer, K. H., Cohen, L. F., Tarone, R. E., Levis, W. L., Kohn, K., Glaubiger, D. G., Slor, H., Popescu, N. C., DiPaolo, J. A., Pizzo, P., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Genetics Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

Psoralen plus long wavelength ultraviolet radiation (UV-A) is being investigated as a model system for clinically relevant photochemical carcinogenesis. Used experimentally for treatment of psoriasis and mycosis fungoides, psoralen plus UV-A

has been found to be mutagenic in bacteria and carcinogenic in mice and humans. We have developed an in vitro assay to measure the effects of psoralen-DNA binding on human lymphoid cells. Parameters monitored include the rate of DNA synthesis, induction of DNA-psoralen cross-links, induced sister chromatid exchanges, alterations in the rate of cell proliferation and survival, and in immune reactivity. We are attempting to measure psoralen-DNA antibodies in rabbits or humans for use as a cellular probe. These studies are aimed at understanding the mechanism of cell damage induced by psoralen plus UV-A so as to minimize the toxicity to human cells during therapy.

BIBLIOGRAPHIC REFERENCES: Kraemer, K.H., Waters, H.L., Ellingson, O.L., and Tarone, R.E.: Psoralen plus ultraviolet radiation-induced inhibition of DNA synthesis and viability in human lymphoid cells in vitro. *Photochem. Photobiol.* 30: 263-270, 1979.

181. ULTRASTRUCTURAL STUDIES OF THE BIOLOGICAL EFFECTS OF ULTRAVIOLET LIGHT AND OF PHOTOACTIVATED 8-METHOXY PSORALEN

Ree, K., Johnsen, A., Norwegian Cancer Society, Dept. of Pathology, Huitfeldtsgt. 49, Montebello, Oslo 3, Norway

OBJECTIVE: To define the ultrastructural alterations taking place in the nuclei of human epithelial cells after photoactivated 8-methoxy psoralen exposure (PUVA). In particular looking for morphological markers that may indicate malignant transformation.

APPROACH: An established human epithelial cell line (NCTC 2544) was exposed to 0.3 microgram 8-methoxy psoralen/ml medium for 30 minutes in the dark and immediately thereafter the cells were irradiated with 1000 mJ/sq. cm UVA ultraviolet light. Cells were fixed and processed for electron microscopy 2 hours, 3 days and 6 days after completing the irradiation. A variety of nuclear parameters were scored and the morphology was compared with control cells fixed and processed at the same time intervals.

RESULTS: An increase in the number of cells with irregular nuclear outline, pseudoinclusions, nuclear bodies and increased number of perichromatin granules were seen after 3 and 6 days. The amount of heterochromatin situated marginally was decreased. Approximately 30-50% of the cells showed several nuclear elements per cell section. Some of these nuclear elements had a bizarre shape.

The ultrastructural changes were compared with the ultrastructural morphology of other epithelial cell lines, both malignant and non-malignant (H.S. Smith, *Cancer Res.* 39, #332-344, 1979). The PUVA exposed cells had several morphological markers in common with the malignant cell lines.

182. THE UVA-PHOTOTOXICITY OF PSORALENS IN RELATION TO THE ROUTE OF ADMINISTRATION WITH PARTICULAR REFERENCE TO CARCINOGENICITY

Jablonska, S., Akademia Medyczna w Warszawie, ul Filtrawa 30, Warsaw, Poland

SCOPE: It is intended to investigate the phototoxic and carcinogenic effects of 8-methoxypsoralen in hairless mice in relation to the route of administration (oral, by stomach tube and intraperitoneal), dosage and accumulation. Detailed investigations will be concerned with the development of cancerous and precancerous changes in the skin and viscera.

Results will be published. The FDA is very interested in evaluating the safety of psoralen UV-A treatments (PUVA) inasmuch as consumer safety is the mission of FDA.

183. CROSS-LINKS BETWEEN STRANDS PRODUCED BY PSORALEN AND NEAR ULTRAVIOLET LIGHT

Howard Flanders, P., Yale University, School of Medicine, Dept. of Therapeutic Radiology, 333 Cedar St., New Haven, Connecticut, 06510, United States of America

This is part of a broader project. A summary of this subproject is not available.

184. MODIFICATION OF CHEMICALLY INDUCED AND SPONTANEOUS CANCERS BY ENVIRONMENTAL AND OCCUPATIONAL STRESSORS (ABBREV)

Szmigielski, S., Bielec, M., Janiak, M., Center for Radiobiology & Radioprotection, 128 Szaserow, 00909 Warsaw, Poland

The aim of this project is to investigate the influence of environmental and occupational stressors (crowding, isolation, light and noise, moderate hypo- and hyperthermia) combined with ionizing and non-ionizing (microwave) radiation on development and growth of experimental cancers. Painting of skin with 3,4-benzopyrene resulting in development of skin cancer will be employed, and strains of mice with high incidence of spontaneous breast cancer (C3H) will be used as experimental models.

Treatment with 3,4-benzopyrene will be applied at different periods prior to or simultaneously with exposition to the above stressors or in combination with radiation. Time of tumor development (7-range macroscopic scale) and survival of animals will be evaluated.

Natural antineoplastic resistance (lung cancer colony test) and natural killer activity in animals exposed for different periods to the above stressors will be also evaluated.

185. COMBINED CARCINOGENIC EFFECT OF RADIATION AND CHEMICALS

Tanooka, H., Hoshino, H., Ministry of Health & Welfare, Radiobiology Div., 3-1-1 Tsukiji, Chuo-ku, Tokyo 104, Tokyo, Japan

OBJECTIVE: To clarify the mechanism of additive carcinogenic effect of radiation and chemicals.

APPROACH: Target is the mouse skin. The skin was irradiated with low-penetrating beta rays in combination with painting with a chemical (e.g., 4-nitroquinoline 1-oxide, 4NQO). Skin tumor formation was then observed.

PROGRESS: With beta rays alone, the effect of dose-fractionation was found. The optimum condition for skin carcinogenesis is a repeated irradiation with 500-1000 rad. Single irradiation with 3000 rad alone did not produce a tumor. However, the latent effect persisted over one year and could be induced by combination with painting with 4NQO of sub-carcinogenic dose.

186. MUTANTS AND ALTERED RADIORESPONSE OF CELLS AND TUMORS

Gregg, E. C., Yau, T. M., Nygaard, O. F., Blaglow, J. E., Jacobson, B. E., Robinson, J. K., Mencl, J., Kim, S. C., Case Western Reserve University, School of Medicine, Dept. of Radiology, 2109 Adelbert Rd., Cleveland, Ohio, 44106, United States of America

Our objectives are severalfold: A. To produce specific single-locus mutations from stable strains of mammalian cells growing in various environments of radiation and in combination with various carcinogenic and chemotherapeutic agents. B. To quantitate the relative and absolute probabilities of mutagenesis for the various agents mentioned above and combinations of same simulating approximately their use in clinical cancer therapy in order to assess the possibility of synergistic action. Very low dose rates of ionizing radiations are also to be used in order to possibly evaluate environmental dose rate effects. C. To quantitate the growth rate of selected mutants of mammalian cells maintained continuously under various concentrations of chemotherapeutic and mutagenic agents. Combinations of these various agents including ionizing radiation and representing typical clinical conditions encountered in cancer therapy are also to be studied for synergistic and/or cytotoxic effects. The effects of very small doses and dose rates of both drugs and ionizing radiations are also to be determined. D. To further identify, describe and assess the biochemical, biophysical and cytological differences between our established lines of radioreistant mutants and their common parental source.

The attainment of these primary objectives will extend our understanding of those factors affecting radiation resistance and sensitization, mutagenesis and tumor growth under various conditions in current and proposed practice for cancer therapy. Lastly, the quantitation of both growth and rates of mutagenesis at very low dose rates can have import not only for highly fractionated therapy involving heat, drugs and radiation but also for the assessment of environmental contaminations.

187. THE BIOLOGIC EFFECTS OF ENVIRONMENTAL RADIATION

Urbach, F., Forbes, P. D., Davies, R. E., Mann, S. S., Berger, D., Logani, M. P., Harper, R. A., Temple University, School of Medicine, Dept. of Dermatology, 3322 Broad St., Philadelphia, Pennsylvania, 19122, United States of America

Studies to be performed include development of high intensity, multiport solar simulators, evaluation of personal UVR dosimeters, photochemistry of environmentally important molecules, biologic effects of UVR on skin and cultured skin cells by itself and with interaction of photochemically active molecules, studies on mechanism of phototoxicity, photoallergy, interaction of light and chemicals, and human phototherapy.

188. CHEMOTHERAPEUTIC AGENTS AND ULTRAVIOLET CARCINOGENESIS

Epstein, J. H., Hoshino, S., University of California, San Francisco Campus, School of Medicine, Dept. of Dermatology, 55, Parnassus Ave., San Francisco, California, 94122, United States of America

The influence of a number of chemotherapeutic agents on the development of cutaneous squamous cell carcinomas and melanomas induced in the hairless mice by ultraviolet energy will be investigated. The chemicals to be studied are primarily cancer chemotherapeutic agents which not only have profound effects on cellular kinetics and melanocyte function but also are being utilized for prolonged periods in non-cancerous diseases. They will be administered systemically or topically depending on the methods utilized in human disease. The formation and growth of the cancers will be monitored. In addition, the acute and chronic effects of these chemicals on DNA synthesis, repair of ultraviolet-damaged DNA, and mitoses formation will be evaluated with radioactive tracer and colcemid techniques. Their influence on various phases of melanocyte function in normal, UV-stimulated, chemical carcinogen-stimulated and malignant melanoma tissue in vivo will be examined by microscopic techniques and dopa staining procedures.

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189. MODIFYING RADIONUCLIDE EFFECTS

Mahlum, D. D., U.S. Dept. of Energy, Battelle Pacific Northwest Lab., P.O. Box 999, Richland, Washington, 99352, United States of America

Potentially important factors studied in this project include the effect of pregnancy and lactation, iron deficiency, alcohol ingestion, and protein deficiency. In addition, a study of the relationships between the size of the exposed area and the effects of beta-irradiation on the skin of miniature swine is being continued under this project.

190. CELL CULTURE STUDIES OF ENVIRONMENTAL PROMOTERS

Baxter, C. S., Christian, R. T., University of Cincinnati, School of Medicine, Dept. of Environmental Health, Eden & Bethesda Aves., Cincinnati, Ohio, 45221, United States of America

Agents, termed promoters, have been identified which, though noncarcinogenic per se, are able, either on mouse skin or systemically, to induce tumorigenesis specifically when administered subsequent to subcarcinogenic doses of chemicals and UV radiation. Furthermore, promoters show this activity even when administered long after carcinogen treatment, at times when DNA repair activity has been suggested to have terminated. Such observations have led to the proposal of an ubiquitous two-stage model of carcinogenesis by chemicals. At present few promoters of environmental relevance have been identified, even though their significance is probably considerable, since they may render tumorigenic doses of carcinogens regarded per se as offering little cancer risk, and whose removal from the environment would be impracticable on physical or economic grounds. The ability of promoters to enhance carcinogen-induced mutation frequencies in cultured V79 cells, discovered in our laboratory, suggests that the latter system has considerable potential in detecting environmental agents with promoting activity. Parallel characteristics of promoter action in animals and in V79 cell culture further suggest that the latter will be valuable for examining the mechanism of action of promoters.

The study will consist of three phases: a) Study of various parameters, including exogenous metabolic activating systems, determining mutation frequencies in the V79 cell mutagenesis system. b) Determination of the effect of tumor-promoting

agents on carcinogen-induced mutation frequencies. c) Determination of the effect of promoters and nonpromoting analogs on biochemical parameters, implicated in tumor promotion, in V79 cells.

191. RADIATION AND CHEMICAL CARCINOGENESIS

Greenstock, C. L., Atomic Energy of Canada Ltd., Medical Biophysics Branch, Pinawa, Manitoba, ROE 1LO, Canada

OBJECTIVE: To understand the chemical effects of radiation on important cell components, and to probe the molecular mechanisms whereby radiation and/or chemical carcinogens produce cancer, using model chemical systems.

APPROACH: Radiation chemical damage to biological molecules, particularly DNA membrane phospholipids and their components, is produced by a ⁶⁰Co gamma ray source, in the presence and absence of chemical carcinogens. The radiation products are isolated and analyzed using HPLC, to determine the mechanism of radiation damage, the involvement of chemical carcinogens, and the extent of any combined or synergistic effects of radiation and chemical carcinogens. Kinetic studies using the technique of pulse radiolysis are being employed to measure the reactivity, selectivity and the nature and extent of involvement, of free radicals, particularly the hydroxyl radical, superoxide anion, singlet oxygen and other forms of activated oxygen, in cancer induction, transformation, or carcinogen activation, at the molecular level.

192. EFFECTS OF PESTICIDES ON DNA SYNTHESIS, REPAIR AND CELLULAR TRANSFORMATION IN MAMMALIAN CELLS CULTURED IN VITRO (ABBREV)

Rocchi, P., Perocco, P., Prodi, G., Università degli Studi di Bologna, Istituto di Cancerologia, via S. Giacomo 14, 40126 Bologna, Italy

OBJECTIVE: To study the effects exerted by pesticides utilized in agriculture on mammalian and human cells cultured in vitro. Particular attention is devoted to the interferences of these chemicals with cellular informative structures.

APPROACH: The parameters considered are: 1) the inhibition of scheduled DNA synthesis exerted by pesticides; 2) the capability of these chemicals to induce damages in DNA molecules measured by tritiated thymidine uptake by cells cultured in the presence of hydroxyurea; 3) their capability of depressing cellular repair processes following damages to DNA exerted by physical agents (i.e. UV or gamma rays); 4) morphological changes and/or transformations of cells cultured with or without pesticides and ultimate carcinogens (i.e., loss of contract inhibition).

PROGRESS: The study of 18 pesticides as agents damaging to human lymphocyte DNA synthesis has shown that: 1) some of them elicit low reparative DNA synthesis; 2) some of them exert an inhibition of DNA reparative synthesis after UV irradiation; 3) lack of correlation between the effects exerted on scheduled and unscheduled DNA synthesis occurs.

193. EFFECT OF CHLOROQUINE ON RADIATION REPAIR IN TUMOR CELLS (HUMAN, MICE)

Laughlin, J. S., Sloan Kettering Inst. for Cancer Research, 410 E. 68th St., New York, New York, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

194. MODIFICATION OF CHEMICALLY INDUCED CANCERS IN ANIMALS WITH LONG-TERM EXPOSURE TO LOW LEVEL MICROWAVE FIELDS (ABBREV)

Szmigielski, S., Szudziński, A., Pietraszek, A., Center for Radiobiology & Radioprotection, 128 Szaserów, 00909 Warsaw, Poland

Rapidly growing environmental and occupational exposure to microwave radiation calls attention to the possible specific (non-thermal) biologic effect of this radiation, including delayed genetic phenomena and the possibility of neoplastic transformation of irradiated cells. Influence of long-term low-level microwave irradiation on cell mediated immune reactions is also postulated. These effects may differ, depending upon frequency of microwaves, time of exposure and power densities used. The following subjects will be elaborated in the project: 1. Development of skin cancer induced by 3,4-benzopyrene in mice ex-

posed for 1-4 months to non-thermal (5 or 15 MW/cm²) power densities of 2450 MHz microwaves (continuous and pulse modulated waves); irradiation with microwaves will be performed prior to or simultaneously with treatment with 3,4-benzopyrene; cancer development will be evaluated macroscopically (7-range scale) and microscopically (routine histology and histochemistry). 2. Development of primary hepatomas in mice injected intraperitoneally with di-ethyl-nitroso-amine /DENA/ and exposed to 2450 MHz microwave field (as above). 3. Natural antineoplastic resistance (cancer lung colonies, natural killer activity) in mice exposed to low level microwave fields.

2. COCARCINOGENICITY OF RADIATION AND VIRUSES

195. STUDIES ON X-RAY-INDUCED LEUKEMIA IN C57BL/6 MICE

Jolicœur, P., Institut de Recherche Clinique de Montreal, Laboratory of Molecular Biology, 110 Ouest Ave. de Pins, Montreal, Quebec, H2W 1R7, Canada

Our recent experiments have provided evidence that X-ray irradiation of C57BL/6 mice induces an ecotropic MuLV that can be isolated during the preleukemic phase as well as in leukemic tissues of the animal. We have also been able to derive several producer lymphoid cell lines from X-ray-induced thymoma.

Our present and future efforts are directed towards the characterization of the viral isolates we have found (host range, leukemogenicity, etc.) and of the cell lines derived.

196. RADIATION AND CHEMICAL EFFECTS ON VIRAL TRANSFORMATION AND TUMOR ANTIGEN EXPRESSION

Coggin, J. H., University of South Alabama, College of Medicine, Dept. of Microbiology, 307 University Blvd., Mobile, Alabama, 36688, United States of America

The objective of this research is to define the role of low level irradiation combined with DNA containing viruses known to exist in the human population to determine the potentiating effects of irradiation on virally induced neoplasms. The second aspect of the work is to define the role of low levels of chemical carcinogens, particularly hydrocarbons, in potentiating the oncogenic properties of viruses. The primary focus of a large portion of the work will center around determining whether or not it is possible to detect the earliest signs of virally or chemically induced tumors, potentiated by hydrocarbons or irradiation through the monitoring of cells or the immune response of the host to tumor associated antigens which appear to be of embryonal or fetal origin. The approach being used is to detect the earliest transformed cells that appear in cultures of cells exposed to cocarcinogens as reflected by their ability to grow on an unusual test culture surface, polyacrylamide, which seems to have the unique property of excluding the growth of normal cells while potentiating the growth of cancer cells. Cells which become adherent to the acrylamide will be monitored for the expression of embryonic antigens which are common to tumors of the same histologic type. The results expected should allow us to determine whether embryonal or fetal immunologic markers can be used to detect the earliest evidence of chemically or virally induced carcinogenesis which can be potentiated by low level irradiation. We anticipate that our results will be in hand in the coming three years.

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197. RADIATION PATHOLOGY AND VIRAL, RADIATION, AND ENVIRONMENTAL ONCOLOGY

Finkel, M. P., U.S. Dept. of Energy, Div. of Biological & Medical Research, 9700 S. Cass Ave., Argonne, Illinois, 60439, United States of America

The program objective is to provide basic information for estimating the cancer risk to man from environmental pollutants

associated with energy production. The historical approach has been to discover where and when following exposure to ionizing radiation the cancer event occurs and to determine how much energy must be absorbed to initiate the oncogenic process. Animal experiments are aimed at (1) defining the true latent period, or time to irreversible neoplastic change, and determining whether it varies with sex, age, species, dose, nuclide, or radiation quality; and (2) locating the microscopic site of neoplastic change, estimating the actual amount of energy delivered to that site, and assessing the influence of dose-rate and exposure pattern on the oncogenic response. Whereas some studies continue in this area, a second approach now receiving most effort is directed toward discovering whether radiation and chemical agents cause cancer by activating endogenous viral information. The virus experiments are aimed at: (1) characterizing the three murine ERP/ANL bone tumor viruses biochemically, biophysically, and biologically, (2) obtaining molecular evidence that sarcoma viruses can be activated by radiation, and (3) determining whether viruses are involved in other spontaneous and induced neoplasms of mice, dogs, and man.

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198. IMMUNOLOGICAL AND VIRAL ASPECTS OF RADIATION-INDUCED TUMORS

Reif, A. E. Mallory Inst. of Pathology Foundation, 818 Harrison Ave., Boston, Massachusetts, 02118, United States of America
Strontium-90 is one of the hazardous products of both peaceful and military use of nuclear energy. While high doses (1 microcurie/g body) are highly carcinogenic in man (on the analogy of the effects of radium), the human population comprises individuals with dissimilar genetics and susceptibility to cancer. The objective of this study is to test strontium-90 carcinogenesis at various doses in mice with different susceptibility to cancer and carrying different types of tumor viruses. Also, the therapy of osteogenic sarcoma by immunological means will be investigated. Strontium-90 carcinogenesis will be studied at different doses in various strains of mice. Mice of a healthy strain will also receive immunosuppression by different means to determine if this will influence the final incidence of tumors induced by strontium-90. Especially whole-body irradiation will be studied in this connection. Immunotherapy trials will be run on tumors induced in inbred mouse strains by strontium-90 and by FBJ Osteosarcoma virus. The relationship between the tumors induced by these two agents will be investigated. An experiment with 701 female C57BL/6 mice is in progress with 19 separate treatment groups. The treatments consisted of various doses of strontium-90, and in addition some groups were given 300 rad total body irradiation, inoculated with active or inactive mouse osteosarcoma virus (FBJ), or immunized with irradiated syngeneic (C57BL/6) osteosarcoma tumor. Results of the experiment to date are reported in our third annual report, and were updated in our contract renewal request dated May 15, 1978, submitted to our contract officer, Dr. George E. Stapleton. The experiment will be completed within 12 months.

199. BIOLOGICAL ASPECTS OF CARCINOGENESIS BY RADIATION

Kaplan, H. S., Decleve, A., Lieberman, M., Stanford University, School of Medicine, Dept. of Radiology, 300 Pasteur Dr., Stanford, California, 94305, United States of America

OBJECTIVES: 1. Continued characterization of RadLV/VL3 structural proteins purified with the aid of monoclonal hybridoma antibodies and studied by SDS-PAGE, by cross-reactivity with antibodies to the corresponding proteins of other murine type C viruses, and by tryptic digestion and high-pressure liquid chromatography of the hydrolysate peptides. 2. Characterization of the temperature-sensitive mutants of RadLV/VL3 recently isolated by Dr. Jean Emeny, with emphasis on their thymotropism and leukemogenicity in vivo,

complementation patterns for replication at nonpermissive temperatures in vitro, and attempts to identify whether a structural protein or the viral reverse transcriptase is the temperature-labile gene product. 3. Further evidence for the viral etiology of radiogenic lymphomas, as outlined previously. 4. Studies of the pathogenesis of virus-induced and of radiation-induced thymic lymphomas, as outlined previously. 5. Studies of the relevance of virus-impaired immunocompetence in leukemogenesis. 6. Studies of the evolution of autonomy in autochthonous lymphomas.

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200. PROBLEMS IN RADIATION EMBRYOLOGY

Brent, R. L., Jensh, R. P., Wheelock, E. F., Gorson, R. O., Schneiderman, M., Thomas Jefferson University, Jefferson Medical College, Dept. of Pediatrics, 1025 Walnut St., Philadelphia, Pennsylvania, 19107, United States of America

The summary for this project is under entry no. 136.

III. EXPERIMENTAL STUDIES IN RADIATION BIOLOGY

A. BIOLOGICAL EFFECTS OF RADIATION IN EXPERIMENTAL SYSTEMS

1. EFFECTS OF RADIATION ON DNA REPAIR IN HUMAN CELLS

201. INFLUENCE OF INCREASED ENVIRONMENTAL RADIATION ON DNA SYNTHESIS, REPAIR AND SISTER CHROMATID EXCHANGE (ABBREV)

Altmann, H., Gunther, R., Egg, D., Pohl Ruhling, J., Fischer, P., Österreichische Studiengesellschaft für Atomenergie G.m.b.H., Inst. für Biologie, A2444 Seibersdorf, Austria

The capacity to repair DNA lesions is tested in lymphocytes of personnel working in a former gold mine, now used for therapeutic purposes, containing an average of 3 nCi/l of ²²²Rn. In addition, unscheduled DNA synthesis is measured in lymphocytes of patients suffering from osteoarthritis and ankylosing spondylitis, respectively, and treated by curative baths with 40 nCi/l ²²²Rn or by desecation to the above mentioned mine. DNA repair is demonstrated by the following methods: a) chromatography of DNA and BND cellulose columns and measurement of ³H-thymidine incorporation into native and single-strand containing DNA fractions b) determination of unscheduled DNA synthesis by autoradiography c) demonstration of reconstitution of supercoils within DNA after irradiation by nucleoid centrifugation.

Further experiments are performed to clarify whether poly-ADP-ribose is synthesized in greater amount after repeated exposure to low radiation doses, thus causing the observed increase of DNA repair capacity in cells of exposed persons.

Besides measuring DNA repair the number of sister chromatid exchanges is evaluated in the above mentioned test persons. Preliminary experiments show a decreased rate of spontaneously occurring sister chromatid exchanges in cells of exposed persons.

202. COORDINATED COMPARATIVE STUDIES ON RADIATION-INDUCED CHROMOSOME ABERRATIONS IN MAN

Schwarzacher, H. G., Universität Wien, Histologisch Embryologisches Inst., Schwarzschanerstr. 17, 1090 Vienna, Austria

The aim of the project is to provide standards for radiation damage to human chromosomes. Lymphocyte cultures stimulated by PHA are used, as well as standard X-ray doses between 100 and 300 rad. Chromosome type and chromatid type aberrations are scored in otherwise untreated and BUdR-treated cultures. BUdR is used to distinguish between first and subsequent

mitoses. The onset of first mitoses will be studied in more detail as before. Also tests on the heterogeneity of the lymphocyte population (early versus late starting lymphocytes) are carried out. One of the studies deals with the effort of the international group of scientists working on the same problems to establish standards of scoring chromosome preparations for aberrations.

203. PRODUCTION OF CHROMOSOME ABERRATIONS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES BY RADIATION THERAPY (ABBREV)

Buckton, K. E., Alexander, L., Medical Research Council, Clinical & Population Cytogenetics Unit, Crewe Rd., c/o Western General Hospital, Edinburgh EH4 2XU, Scotland, United Kingdom

It has been shown, by a number of centers, that radiation induced chromosome damage can be detected in the peripheral blood lymphocytes of an irradiated person many years after the exposure to irradiation. In the present study, a population of patients suffering from ankylosing spondylitis, who have been exposed to a single course of X-ray treatment (of between 1500-2500 rad to the whole spine and sacroiliac joints) is being studied in order to evaluate (1) the persistence in the body of cells with radiation induced asymmetrical and symmetrical chromosome exchanges; (2) the points on the chromosomes at which the exchanges occur, and whether there is a selection for particular exchange breakpoints; (3) the development of clones of cells with the same structural rearrangement in the blood of irradiated individuals and (4) the development of any late effects from the irradiation, particularly cancer, in these patients on whom cytogenetic data is available.

Peripheral blood will be taken from a population of patients with ankylosing spondylitis known to have had only one course of treatment at least four years previously. Part of the sample will be used for a routine hematological blood count and the rest for the cytogenetic study. Where possible 50-100 G- and/or R-banded cells from each patient will be fully karyotyped on the light microscope.

Details of the patients' medical history, HLA phenotype, smoking habits and occupation will be collected in case this should have any bearing on the patient's morbidity.

In addition, the senior investigator (KEB) will take part in the efforts to standardize the scoring of chromosome aberrations to help to develop the necessary criterion for a biological monitoring system.

204. EFFECTS OF RADIATION ON STATIONARY CELLS

Little, J. B., Reynolds, R., Zamansky, G. B., Nagasawa, H., Weichselbaum, R. R., Harvard University, Boston Campus, School of Public Health, Dept. of Physiology, 55 Shattuck St., Boston, Massachusetts, 02115, United States of America

The long-term goal of this research program is to gain knowledge about mechanisms which determine the radiosensitivity of mammalian cells, particularly those involved in the response of stationary or very slowly proliferating cells to X-irradiation. We will utilize three different types of cells: 10T1/2 mouse embryo fibroblasts, human diploid cells and human tumor cells. Four cellular endpoints will be studied in parallel: survival, malignant transformation, mutagenesis, and the induction of sister chromatid exchanges (SCE). We will investigate the relationships between these cellular effects, and try to correlate them with molecular DNA repair processes. Techniques for measuring DNA repair will include host cell reactivation of irradiated herpes virus, alkaline elution assay for DNA strand breaks and cross-linking, and measurement of endonuclease sensitive sites.

Cellular and molecular phenomena will be studied during the repair of potentially lethal damage (PLD) in stationary phase 10T1/2 and human diploid cells treated with X-rays, UV light or mitomycin-C. The frequencies of induced mutations, SCE and malignant transformation will be studied in parallel experiments, and correlated with molecular repair. In particular, evidence will be sought which implicates the action of error-prone repair processes in the cellular effects induced by these agents. Further information on mechanisms will be obtained through the study of skin fibroblasts isolated from individuals who are unusually susceptible to the development of cancer, especially radiation induced cancer. We will measure the sensitivity of these cells to the mutagenic effects of radiation in vitro

(mutations to 8-azoguanine and ouabain resistance), and seek evidence for molecular DNA repair defects. Emphasis will be placed on the study of fibroblasts from the hereditary and sporadic forms of retinoblastoma, as well as retinoblastoma heterozygotes. The effects of SV-40 transformation on repair deficient strains will be investigated. Finally, rapid split dose recovery and PLD repair will be studied in plateau phase human tumor cell lines. In particular, the unusual UV sensitivity and X-ray PLD repair capacity we have identified in osteosarcoma cells will be further investigated.

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205. MECHANISMS IN SHOULDERED SURVIVAL CURVES

Ward, J. F., Byfield, J. E., University of California, San Diego Campus, School of Medicine, Dept. of Radiology, P.O. Box 109, La Jolla, California, 92093, United States of America

Three separate hypotheses are presented for a molecular description of the events which occur to produce a shouldered radiation survival curve. (1) The lethal event is a double-strand break, the yield of which increases with post-irradiation time due to hydrolysis of labile bonds. With increasing dose, the repair system becomes saturated and more potential breaks become actual breaks, hence the shoulder. In this case, Elkind-Sutton repair is the complete expression of the potential damage. (2) Sulfhydryl compounds, active in chemical repair, are depleted by increasing radiation doses and cells become more sensitive. Non-protein sulfhydryls and/or protein bound sulfhydryls could be involved. Here Elkind-Sutton repair is the re-establishment of pre-irradiation SH levels. (3) A significant repair enzyme, DNA polymerase, is consumed by binding irreversibly to radiation damaged sites on DNA. With increasing dose, less repair is possible so cells become more sensitive. Again, Elkind-Sutton repair is re-establishment of pre-irradiation polymerase levels.

Experiments are proposed to test these hypotheses both in vitro - aqueous solution radiation chemistry - and in vivo - using cells which have characteristic shouldered response HeLa and CHO.

206. NATURE AND REPAIR OF A NEW FORM OF DNA DAMAGE

Feldberg, R. S., Tufts University, Medford Campus, School of Liberal Arts, Dept. of Biology, Medford, Massachusetts, 02155, United States of America

A damage-specific DNA binding protein from human cells will be employed as a probe for nucleotide damage in DNA. This protein recognizes lesions in DNA treated with UV light or X-rays, as well as in HNO₂, NaHSO₃, H₂O₂- or OsO₄-treated DNA. The protein does not bind, however, to apurinic sites, single-strand breaks, single-strand regions, thymine dimers or psoralen-induced crosslinks in DNA. Thus, the protein functions as a broad-specificity probe for monofunctional base damage in DNA. In this work, we will further characterize this protein and use it to follow damage introduction into DNA treated with a variety of mutagenic or carcinogenic agents. In addition, a variety of genetic disorders characterized by abnormal sensitivity to radiation will be screened for the presence of this protein.

207. UV TRANSFORMATION AND DNA REPAIR IN HUMAN CELLS AND SKIN

Sutherland, B. M., Farland, W. H., Brookhaven National Lab., Upton, New York, 11973, United States of America

We have developed a system for ultraviolet light (200 nm less than lambda less than 400 nm) transformation to anchorage-independent growth of human cells in culture. We shall characterize the transformation process wavelength dependence, influence of age and origin of cells, effect of irradiation and growth schedule, as well as the role of DNA and/or misrepair. We shall characterize the growth patterns, efficiency of anchorage-independent growth, karyotype and chromosomal banding patterns and tumorigenicity of the transformants.

We shall use alkaline agarose gel techniques to detect low levels of DNA damage and repair in small quantities of non-radioactive human fibroblasts, leukocytes and skin. We shall obtain action spectra for pyrimidine dimer formation in human skin and compare it to those for ultraviolet oncogenesis in the mouse. We shall determine rates of repair of dimers by excision and by photoreactivation in human skin.

We shall determine the relationship between UV transformation in vitro and solar oncogenesis in human skin, and the possible roles of DNA repair or misrepair in these processes.

208. GROWTH, NUTRITION, AND NEOPLASTIC TRANSFORMATION OF MAMMALIAN CELLS IN VITRO

Taylor, W. G., Sanford, K. K., Gantt, R. R., Parshad, R., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., In Vitro Carcinogenesis Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

The long-term objective of this program is to understand the mechanism(s) of neoplastic transformation in cultured mammalian cells, particularly human cells. Studies described address two intermediate goals: (a) the role of calcium and dissolved oxygen on survival, proliferation, and differentiated function; and (b) the induction of cellular injury after cell exposure to low-intensity visible light. In studies to date, a lowered calcium concentration appears to retard differentiation of human epithelial cells in that squame formation is not observed; unlike the mouse epithelial system, however, proliferation is not enhanced by lowering calcium concentration. In addition, human skin and monkey kidney cells exhibit a critical oxygen requirement for attachment and proliferation, and monkey kidney cells use oxygen more rapidly than fibroblasts at the same cell density. This rapid utilization affects proliferation and functional responses. Visible light causes chromatid damage in normal human fibroblasts. A medium-specific, cell-type specific cell killing is observed when fibroblasts are exposed to light in medium NCTC 168, a response mediated by hydrogen peroxide products during light exposure.

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209. CELL IRRADIATIONS WITH MOLECULAR IONS

Rossi, H. H., Hall, E. J., Bird, R. P., Geard, C. R., Goodman, L., Lam, Y. P., Rohrig, N., Columbia University, College of Physicians & Surgeons, Dept. of Radiology, 630 W. 168th St., New York, New York, 10032, United States of America

Pairs or triplets of charged particles separated by distances of a few hundred nanometers are more effective at both cell killing and the induction of chromosome aberrations than randomly incident particles. The effectiveness of these associated ions decreases as the particle separation increases in a way that sheds light on the interaction of sub-lesions that leads to the observable endpoints of cell lethality and aberration production. Future experiments will be designed to extend previous studies to include T1 cells of human kidney origin and a human radiation-sensitive ataxia telangiectasia cell line. Attempts will also be made to determine the effects of associated ions in the incidence of oncogenic transformations in C3H 10T1/2 cells in order to shed light on the mechanism of radiation carcinogenesis.

The principal objective of these experiments is to employ pairs or triplets of particles produced by a molecular ion beam to determine the effects of spatial distribution of absorbed radiation energy on cell killing, chromosome damage and cell transformation. The experiments should provide information on the distribution of DNA in the interphase nucleus.

BIBLIOGRAPHIC REFERENCES: Kellenger, A.M. and Rossi, H.H. A Generalized Formulation of Dual Radiation Action. *Radiat. Res.* 75, 471-488, 1978.

210. CELLULAR REPAIR OF POTENTIALLY MUTAGENIC DAMAGE

Ward, J. F., Lewis, H. L., University of California, Los Angeles Campus, Lab. of Nuclear Medicine & Radiation Biology, 900 Veteran Ave., Los Angeles, California, 90024, United States of America

The objectives of this research are: (1) to develop highly sensitive serologic assays for DNA base damage, (2) to apply the assays to the study of cellular damage and its repair, and (3) to study the relation of base damage to carcinogenesis and mutagenesis. The serologic approach is important since current physical-chemical methods for the study of base damage at low doses and within the DNA macromolecule are inadequate. Serologic assays are highly sensitive and specific and permit quantitation of particular ionizing radiation damaged bases. Assay systems are being developed for quantitation of products in isolated DNA and for localization within cell preparations.

Antibodies have been prepared to the ionizing radiation products, 3-hydroxymethyluridine and 8,5'-cycloadenosine, and by phage neutralization assay yields have been measured in irradiated solutions of parent compounds under several conditions. Both products have been detected in irradiated DNA. We have developed a radioimmunoassay for UV photoproducts and production and repair of damage in HeLa cells has been followed. Antibodies have been prepared to O6-ethylguanosine and a highly sensitive method for the detection of this product in ethylnitrosourea treated DNA has been developed.

Repair studies will be extended to include ionizing radiation and chemical carcinogen damaged bases. Damage accumulation and repair will be correlated with survival and mutagenesis in cell systems.

211. PHOTODYNAMIC DNA DAMAGE AND REPAIR IN HUMAN CELLS

Hariharan, P. V., Roswell Park Memorial Inst., Dept. of Biophysics, 666 Elm St., Buffalo, New York, 14263, United States of America

The two main objectives of the proposed research are: (1) to explore possibilities of improving the present methods of phototherapy of human tumors by studying the basic mechanisms of cell killing using different dye-light combinations and, (2) to accomplish a better understanding of the role of DNA damage and repair in photodynamic carcinogenesis. The present studies will concentrate on an anionic porphyrin, such as hematoporphyrin (Hpd), and, a cationic porphyrin such as the tetraiodide salt of meso tetra (4-N-methyl pyridyl) porphine (4-MPyP), to be used as photodynamic sensitizers in human cells grown in culture. The production of hydroxyl radicals will be measured in human cells that have been exposed to various concentrations of the porphyrins and different levels of light. This will be achieved by determining the yield of (1) thymine glycols in the DNA radioactively labeled in the thymine moiety (methyl-3H).

DNA damage and repair of dye-light treated normal human cells, grown in culture, will be analyzed by measuring single strand breaks and rejoining, using the very sensitive and powerful alkaline elution technique. Such studies will be extended to cancer-prone cells, such as xeroderma pigmentosum, in order to understand the role of DNA repair in photodynamic carcinogenesis.

212. PHOTO-INDUCED CELL KILLING, MUTATION, AND DNA REPAIR

Ritter, M. A., University of Pennsylvania, School of Medicine, Dept. of Radiation Therapy, 36th & Hamilton Walk, Philadelphia, Pennsylvania, 19174, United States of America

This study is investigating photochemically-induced cell lethality and mutagenesis (6-thioguanine resistance) in normal and xeroderma pigmentosum human diploid cells as a function of wavelength. Selected wavelengths in the far UV, near UV, and visible range are being used.

We are investigating the role of DNA damage and repair phenomena in cell lethality and mutagenesis. At each wavelength, DNA strand breaks, pyrimidine dimers, excision resynthesis, and the average size of repair-synthesized DNA regions will be determined. The oxygen dependence of cell lethality, mutagenesis, and DNA damage and repair is also being measured as a function of wavelength. This will allow determination

of the contribution of direct and photodynamic damage mechanisms to these radiation effects.

The overall objectives of this study are to measure lethality and mutagenesis induced by radiations between 254 and 550 nm, and to investigate the basic mechanisms involved.

213. DNA INSULT INDUCED BY BUDR AND IRRADIATION (RATS, HAMSTERS, HUMAN)

Ts'o, P. O., Johns Hopkins University, School of Arts & Sciences, Dept. of Biophysics, 34th & Charles Sts., Baltimore, Maryland, 21218, United States of America

This is part of a broader project. A summary of this subproject is not available.

2. EFFECT OF RADIATION ON DNA REPAIR IN PERSONS WITH HEREDITARY DISORDERS

214. PURIFICATION AND CHARACTERIZATION OF HUMAN ENZYMES FOR REPAIR OF DAMAGES TO DNA

Oikawa, A., Tohoku University, Research Inst. for Tuberculosis & Cancer, Dept. of Pharmacology, 4-1 Seiryomachi, Sendai-shi 980, Miyagi-ken, Japan

OBJECTIVE: Enzymes for base excision and nucleotide excision repairs of damaged DNA will be purified from cultured human lymphoblastoid cell lines, which have been established from normal donors and xeroderma pigmentosum patients. At the same time, catalytic properties of crude cell-free extracts and partially purified enzymes will be examined with UV-damaged and chemically modified DNA, and their reconstituted chromatins.

APPROACH: Cell lines to be used are those reported in Cancer Research, 38, 253-256; 38, 3560-3562 (1978). Reconstituted chromatins modified at DNA bases are prepared by salt gradient dialysis of the mixtures of calf thymus chromosomal protein and DNA's (PBS 1 phage DNA, UV-irradiated DNA, DNA's alkylated with various alkylating agents). As a routine test for degree of intactness of reconstituted chromatins, micrococcal nuclease digestion will be carried out.

215. DNA REPAIR PROCESSES IN XERODERMA PIGMENTOSUM

Andrews, A. D., Columbia University, College of Physicians & Surgeons, Dept. of Dermatology, 630 W. 168th St., New York, New York, 10032, United States of America

Mutations in at least eight genes affecting the repair of ultraviolet-induced DNA damage have been identified among patients with xeroderma pigmentosum. The proposed studies will compare the responses of cells from normals and from patients with these different forms of xeroderma pigmentosum to ultraviolet damage. The studies have five interrelated objectives: 1) To determine the relationship between DNA repair capacity and the cell cycles, by studying colony-forming ability following ultraviolet radiation in synchronized fibroblast cultures derived from patients with xeroderma pigmentosum. 2) To identify the portion of the UV spectrum (i.e., the action spectrum) responsible for the photochemical damage to which patients with xeroderma pigmentosum are susceptible. This will be accomplished by studying cell survival following irradiation with different ultraviolet sources. 3) To assess the possible interactions of different wavelengths of UV in the repair processes of cells from normal and xeroderma pigmentosum patients, by comparing the effects of combined and separate irradiations with different portions of the UV spectrum. 4) To assess the role of 'inducible' (and presumably mutagenic) repair processes in cells of normal and xeroderma pigmentosum patients, by seeking to demonstrate an enhanced survival of ultraviolet-irradiated cells following the administrations of small 'inducing' doses. 5) To continue the identification and characterization of new repair mutations in xeroderma pigmentosum patients, by determining repair synthesis rates, complementation groups, and ultraviolet sensitivity.

The overall goal of these studies is to increase our understanding of the relationship between human DNA repair and environmentally-induced diseases of the skin and other organs.

216. THE RELATIONSHIP BETWEEN CARCINOGENESIS AND DNA REPAIR IN XERODERMA PIGMENTOSUM HETEROZYGOTES

Ritter, M. A., University of Pennsylvania, School of Medicine, Dept. of Radiation Therapy, 36th & Hamilton Walk, Philadelphia, Pennsylvania, 19174, United States of America

Ultraviolet light-induced DNA repair rates will be measured in a number of fibroblast lines that are obligate heterozygotes for xeroderma pigmentosum (XP). XP is a skin cancer prone, autosomally recessive human disease. Homozygotes for XP are defective in repairing UV-induced DNA damage.

Our preliminary results, obtained using an M. luteus endonuclease assay for DNA repair, indicate reduced repair, relative to normals, in all 8 obligate XP heterozygote lines that we have tested. In addition, a reduced repair rate was found in one unaffected sibling of an XP family. These heterozygotes include representatives of complementation groups A through D, as well as several unclassified lines.

We will extend our study to 15-20 additional heterozygote lines. We will measure: excision resynthesis with BND-cellulose chromatography, removal of endonuclease-sensitive sites, repair of N-acetoxy-2-acetyl-aminofluorene (AAAF) induced DNA damage and, in obligate heterozygote parents of XP 'variants', we will measure levels of post-replication repair.

Our goals are to: 1) further document the existence of DNA repair deficiencies in XP heterozygotes; 2) compare repair rates in heterozygotes with skin cancer frequencies in blood relatives. This skin cancer data is now available from studies by Dr. M. Swift of skin cancer frequencies in XP families; and 3) investigate aspects of the genetic complexity of XP through analysis of intrafamilial variations in repair. Our results may promote a clearer understanding of the role of DNA repair genes in human carcinogenesis.

217. DNA REPAIR IN HUMAN CANCER-PRONE GENETIC DISEASES

Kraemer, K. H., Scudiero, D. A., Levis, W. L., Greene, M. H., Kohn, P. H., Stinson, S. F., Cohen, L. F., Gelboin, H. V., Bader, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Genetics Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

Human cancer-prone genetic diseases are being studied in order to identify groups of people with an increased susceptibility to environmental carcinogenesis, and to attempt to correlate such sensitivity with clinical features and with defective DNA repair. Patients with xeroderma pigmentosum and ataxia telangiectasia, diseases with ultraviolet and X-ray sensitivity, respectively, and with familial malignant melanoma (dysplastic nevus syndrome), and neurofibromatosis, diseases with no documented environmental sensitivity, are being studied. Detailed examinations of the clinical features of affected individuals are being made. Cultures of skin and blood are being established and the effects on cell survival, DNA repair, and chromosome abnormalities after treatment with DNA damaging agents (ultraviolet, bleomycin, and psoralen plus long wavelength ultraviolet) are being examined.

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218. STUDIES OF DNA REPAIR IN HUMAN DEGENERATIVE DISEASES

Robbins, J. H., Moshell, A. N., Scarpinato, R. G., Snowden, K. I., Potkay, S., Tarone, R. E., Doniger, J., Scudiero, D. A., Lytle, C. D., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Dermatology Branch, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

Studies in this laboratory are designed to elucidate the role of DNA repair processes in human diseases and in carcinogenesis, mutagenesis, and normal and abnormal aging. Most studies have been conducted with cells from patients with xeroderma pigmentosum (XP), all of whom have defective DNA repair plus the clinical abnormalities of multiple cutaneous malignancies and premature aging of sun-exposed skin. Studies are currently being performed on cells from patients with degenerative

neurological diseases such as Huntington's disease, ataxia telangiectasia, familial dysautonomia, olivopontocerebellar atrophy, and Cockayne's syndrome. These studies are designed to elucidate the pathogenesis of these disorders as well as to develop presymptomatic (including prenatal) diagnostic tests. We assess the biological effectiveness of DNA repair primarily by *in vitro* assays of post-irradiation colony-forming ability and trypan blue exclusion. We are extending our work to include cells from patients with other degenerative disorders including Shy-Drager syndrome, idiopathic orthostatic hypotension, retinitis pigmentosa, Usher's syndrome, spinal muscular atrophy, and muscular dystrophy.

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219. DNA REPAIR MECHANISMS IN HUMAN CELLS

Hanawalt, P. C., Smith, C. A., Ganesan, A. K., Stanford University, School of Humanities & Sciences, Dept. of Biological Sciences, Stanford, California, 94305, United States of America

Our principal objective is to understand the role of DNA repair processes in carcinogenesis. Our research is relevant to an understanding of hereditary diseases, such as xeroderma pigmentosum (XP), in which DNA repair is defective. DNA repair in ultraviolet (UV) irradiated or carcinogen treated human cells is being studied by the following approaches: (a) Exogenous substrates and enzymes are added to permeabilized cells to learn details of excision repair. We find UV-dependent stimulation of repair replication upon addition of the T4 endonuclease V to XP nuclei. Other nucleases and polymerases will be added. Other XP complementation groups and genetic defects will be examined. (b) Regions of chromatin accessible to damage and repair are being examined in UV irradiated or psoralen-UV treated cells, utilizing the differential sensitivity of nucleosome cores to staphylococcal nuclease. Bound psoralen and extent of repair replication are to be compared with the UV results. (c) UV irradiated SV40 is used as a probe for detecting inducible DNA repair in mammalian cells. Having shown survival enhancement in carcinogen treated host cells we are now examining intermediates in SV40 DNA replication following UV irradiation. (d) Repair of angelicin (monoadducts) and psoralen (monoadducts and crosslinks) is being compared in W138 and XP cells. (e) DNA polymerization on templates containing UV-induced pyrimidine dimers is to be examined using purified polymerases lacking 3'-5' proofreading exonuclease activity. (f) Fusions between complementing XP cell lines and mouse cell lines are being carried out. Hybrids are to be tested for karyotype and repair properties to map repair genes in the human genome. A search for repair-deficient mutants is to be carried out to aid the analysis of DNA repair pathways in human cells.

220. CELL RESPONSE TO DNA DAMAGE BY UV AND OTHER MUTAGENS

Carter, D. M., Cohen, S. R., Wayne, S., Gala, M., Yale University, School of Medicine, Dept. of Dermatology, 333 Cedar St., New Haven, Connecticut, 06510, United States of America

Our objective in this work is to study factors that contribute to human susceptibility to cutaneous malignancy. We want to know why some persons with certain heritable disorders and some who have been exposed to environmental mutagens develop more skin cancers than normal. We are concerned with the long-term and short-term biological consequences of photomediated, covalent psoralen-DNA photoadducts such as are produced by photochemotherapy (PUVA) with 8-methoxypsoralen (8-MOP) and UV-light (365 nm).

After *in vitro* binding by UV-A of 4,5,8 trimethylpsoralen (TMP) and 8-MOP we are comparing cutaneous cells and lymphocytes from persons in normal and disease states with respect to cell growth and survival, and repair of psoralen DNA crosslinks. We are using cells from persons with dyskeratosis congenita and xeroderma pigmentosum and from their relatives. We are also measuring concentrations of psoralens in blood of our PUVA patients and correlating these values with the patients'

phototoxic and therapeutic responses. We are comparing the cross-linking capabilities of several different psoralens.

BIBLIOGRAPHIC REFERENCES: Carter, D.M., Pan, M. and Varga, J. Pigment Response of Melanoma Cells to Psoralens and Light. Proceedings of the Xth International Pigment Cell Congress, Karger, Basel, 4: 329, 1979. Cassuto, E. and Carter, D.M. Interstrand Cross-links in DNA of Phage Lambda after Exposure to 8-Methoxypsoralen and Trimethylpsoralen in the Presence of Light. *J. Invest. Dermatol.* 73: 236-238, 1979.

221. DNA REPAIR MECHANISMS IN CARCINOGENESIS

Regan, J. D., U.S. Dept. of Energy, Oak Ridge National Lab., P.O. Box X, Oak Ridge Operations Office, Oak Ridge, Tennessee, 37830, United States of America

OBJECTIVES: To elucidate the molecular events in human cells when cellular macromolecules such as DNA are damaged by radiation or chemical agents by studying and characterizing the sequence of DNA repair events, the various modalities of repair, the genetic inhibition of repair due to mutation, and the physiological inhibition of repair due to biochemical inhibitors.

MAJOR FINDINGS: Normal human cells are able to repair ultraviolet (UV) light induced pyrimidine dimers in their DNA while cells derived from patients with the genetic disease Xeroderma pigmentosum (XP) show defective or reduced repair. The number and kinds of pyrimidine dimers formed in DNA by ultraviolet light is related to the DNA base ratio, the wavelength and the total exposure to UV. After 50 J/m² at 254 nm the ratio of dimers (C greater than less than C, C greater than less than T, T greater than less than T) in human cellular DNA was 14:20:66 (24:38:38 in *E. coli*). A low dose of an FS40 sunlamp resulted in a ratio of 20:40:40. Normal human cells approximately 10 to the 6th power dimers within a 24 hour period after 254 nm or FS40 irradiation. About one million dimers were formed in cells by 10 J/m² of 254 nm or 10 to the third power J/m² of filtered sunlamp. At low doses nearly all of the dimers were excised. At higher doses dimer excision was inhibited.

Arabinofuranosyl cytosine (ara-C) at 20 micron m concentration in the presence of 2 mM hydroxyurea has been found to inhibit steps in DNA excision repair following UV irradiation and chemical insult. It causes gaps to accumulate in incised regions which can be observed on alkaline sucrose gradients. Whereas cells from XP patients show a decrease in strand interruptions following UV irradiation in the presence of ara-C (reflecting their lower excision repair capacity), cells from variant lines of XP show an increase in strand interruptions relative to normal human cells. In studies to elucidate the mechanism by which ara-C inhibits repair, it appears that ara-C is capable of at least a two-step inactivation process whereby an initial interaction with the DNA polymerase and/or exonuclease inhibits closure of repair gaps. This is followed by an apparent ara-C dose-dependent inhibition of the incision step. (Text Abridged.)

222. EFFECTS OF RADIATION ON HUMAN CELLS CULTURED IN VITRO

Kantor, G. J., Wright State University, School of Science & Engineering, Dept. of Biological Sciences, Dayton, Ohio, 45431, United States of America

The proposed research is a study of the effects of low doses (sublethal) and repeated exposure to low doses of ultraviolet (UV, 254 nm) and ionizing radiation (X-rays) on cultured human diploid fibroblasts (HDF). Current emphasis is on defining the effects of these radiations on arrested nonmitotic populations of various HDF strains including the DNA repair-deficient xeroderma pigmentosum (XP) and ataxia telangiectasia (AT) strains and determining the rate and extent of DNA repair in these arrested populations. Another emphasis is on inducing with these radiations HDF transformants in culture.

A fraction of cells from UV-irradiated arrested populations is lost upon subsequent incubation. The extent of cell loss is dose dependent and cell strain specific. Xeroderma pigmentosum strains are more sensitive to UV than are normal HDF. The sensitive target is DNA. The lethal event appears to be one on RNA transcription that leads to an inhibition of required protein synthesis. DNA excision repair proficient strains are more resistant to UV because they can remove to some extent the DNA damage that leads to an effect on RNA synthesis.

Experiments to study the rate and extent of DNA repair in both UV and X-irradiated normal, XP, and AT populations of nondividing cells are proposed. The assay for repair is the loss of DNA sites sensitive to UV-specific or gamma-specific endonuclease. The objective is to gain from these rate studies an understanding of the mechanisms of excision repair in normal cells and some insight into the repair defects in the other strains.

Other experiments to induce and detect HDF transformants in culture are proposed. Emphasis is placed on using HDF strains isolated from individuals with a predisposition to cancer, using fresh primary isolates, using single and multiple exposures to X-rays and UV with chemicals defined as cocarcinogens (phorbol esters, diethylstilbestrol, anthralin) and culture procedures that permit expression of a transformation event.

BIBLIOGRAPHIC REFERENCES: Kantor, G.J., R.S. Petty, C. Warner, D.J.H. Phillips and D.R. Hull. 1980. Repair of radiation-induced DNA damage in non-dividing human diploid fibroblasts. *Biophys. J.*, in press. Kantor, G.J., J.C. Sutherland and R.B. Setlow. 1980. Action spectra for killing non-dividing normal human and xeroderma pigmentosum cells. *Photochem. Photobiol.*, in press.

223. CYTOTOXIC, MUTAGENIC AND CARCINOGENIC EFFECTS OF ENERGY RELATED AGENTS IN DIPLOID HUMAN CELLS WHICH DIFFER IN DNA REPAIR CAPACITY

McCormick, J. J., Michigan State University, School of Osteopathic Medicine, Dept. of Osteopathic Medicine, *Soil Science Bldg., East Lansing, Michigan, 48824, United States of America*

A quantitative system has been perfected to measure the cytotoxicity of chemicals and radiation in diploid human cells in culture and the frequency of induced mutations. The system has been adapted to diploid cells derived from persons with genetic predisposition to cancer and defective capacity for DNA repair. Studies to date indicate a direct relationship between the cells' capacity to repair damage to DNA and the cytotoxic and mutagenic response and demonstrate that, in contrast to the response of commonly employed aneuploid mammalian cell lines, no measurable increase in mutations is induced by several agents at doses which yield a shoulder on the survival curves of normally repairing cells. Using diploid human fibroblasts with different capacities for DNA repair, we will: (1) Investigate the relationship between threshold doses for cytotoxicity and mutagenicity and the ability of cells to repair lesions in DNA caused by low doses of energy-related chemicals and radiation; (2) quantitate the number and kind of chemical adducts remaining bound to the DNA of cells at critical times following exposure to determine the correlation between these adducts and the cytotoxic and mutagenic effects of the chemicals; (3) measure the cytotoxic effect of selected industrial carcinogenic metals in human cells and determine whether they can induce mutations and/or transformation; and finally, (4) investigate the cytotoxic and mutagenic effect of these energy-related agents in diploid fibroblasts derived from a series of selected mammals in order to compare the results with those determined above.

BIBLIOGRAPHIC REFERENCES: McCormick, J.J., and V.M. Maher, *Mammalian Cell Mutagenesis as a Biological Consequence of DNA Damage*, In *DNA Repair Mechanisms*, P.C. Hanawalt, E.C. Friedberg and C.F. Fox (eds.), Academic Press, New York, 1978.

224. CARCINOGEN DAMAGE AND REPAIR OF DNA IN HUMAN CHROMATIN

Smerdon, M. J., Washington University, School of Medicine, Dept. of Pathology, 660 S. Euclid Ave., St. Louis, Missouri, 63110, United States of America

The broad objective of this study is to understand the role of chromatin structure in the distribution of carcinogen-induced damage to DNA and its subsequent repair in human cells. In summary of the present report, DNA repair synthesis within different structural regions of chromatin was measured by a mathematical analysis of the kinetics of digestion with staphylococcal nuclease and DNase I. In addition gel electrophoresis was used to analyze DNA fragments resistant to these enzymes. It was found that UV-induced DNA repair synthesis in cultured human fibroblasts occurs almost entirely in the linker regions of nucleosomes. Following repair synthesis, 80-85% of the repair-

incorporated nucleotides are involved in nucleosome rearrangement yielding a more uniform distribution of repair synthesis in linker and core DNA. A small fraction of the repair-incorporated nucleotides (15-20%) are not involved with this rapid rearrangement and remain nuclease sensitive. Neither the distribution of repair synthesis nor the rate and extent of rearrangement were affected by different UV doses (1.5-50 J/m²), the presence of hydroxyurea, or the rate of repair synthesis. Similar results were obtained with two different classes of xeroderma pigmentosum (XP) cells which have been shown to be repair deficient.

225. REPAIR OF CARCINOGEN-INDUCED DNA DAMAGE IN HUMAN CELLS - THE ROLE OF ADP RIBOSYLATION

Lieberman, M. W., Cassel, A. F., Washington University, School of Medicine, Dept. of Pathology, 660 S. Euclid Ave., St. Louis, Missouri, 63110, United States of America

The overall objective of our work is to understand more about the way in which human cells repair DNA damage induced by carcinogens. The aim of this research program is to investigate the possible relationship between ADP ribosylation and DNA damage and repair. We plan to evaluate the role of ADP ribosylation in DNA repair in human cells damaged with direct-acting chemical carcinogens and ultraviolet radiation. Chemical carcinogens will include: 'short patch', X ray-mimetic agents (e.g., methylnitrosourea (MNU) and methylnitrosoguanidine (MNNG)); and 'long patch', UV-mimetic agents (e.g., N-acetoxy-2-acetylaminofluorene (NA-AAF) and 7-bromomethylbenz (a)-anthracene (BMBA)). If stimulation of ADP ribosylation by long patch agents is confirmed, repair deficient xeroderma pigmentosum cells damaged with these agents will be used to determine whether the stimulation of ADP ribosylation is the result of DNA damage alone or is part of the DNA repair process. Using polyacrylamide gel electrophoresis we will examine ADP ribosylation of nuclear proteins during repair with special emphasis on nucleosome structure and histones H1 and H2B.

226. ENZYMES AND REACTIONS FOR REPAIR OF DNA IN HUMAN CELLS

Brent, T. P., St. Jude Childrens Research Hospital, Dept. of Biochemical & Clinical Pharmacology, 332 N. Lauderdale St., P.O. Box 318, Memphis, Tennessee, 38101, United States of America

Two enzyme activities that act specifically on damaged RNA will be purified from human placenta and characterized with respect to substrate lesion specificity. The first is an endonuclease that acts on UV- or x-irradiated DNA. The second is an N-glycosidase that acts on methylated or ethylated DNA.

The levels of these enzymes will be examined in mutant human cell lines that are characterized both by unusual sensitivity to radiation or alkylation and by elevated incidences of neoplasia. These enzymes will also be used as probes to identify and measure DNA lesions induced by genotoxic agents in normal and mutant human cell lines, with a view to determining efficiencies of repair in such cell lines.

BIBLIOGRAPHIC REFERENCES: Partial purification and characterization of a human 3-methyladenine-DNA glycozylase. Brent, T.P. *Biochemistry* 18:911-916 (1979). 3-methyladenine-DNA glycozylase: A probe for determining alkylation damage and repair in human fibroblasts. Brent, T.P. *Biochem. Biophys. Res. Commun.* 91:795-801 (1979).

227. DNA REPAIR - HUMAN, E. COLI PHOTOREACTIVATING ENZYMES

Sutherland, B. M., Farland, W. H., Beck, L. G., Robison, S. H., Associated Universities Inc., Dept. of Biology, 1717 Massachusetts Ave. N.W., Washington, District of Columbia, 20036, United States of America

Photoreactivating enzymes (PREs) play a unique role in DNA repair by their specific, light-dependent monomerization of cyclobutyl pyrimidine dimers in DNA. We shall determine the structure of the E. coli PRE, both apoprotein and 'cofactor', and study their relation to the binding and photolytic reactions. We shall isolate large quantities of the mammalian PRE and determine its structure.

The specificity of the enzyme allows its use as a dimer probe: if detrimental biological effects of UV can be reversed in a true photoenzymatic reaction, dimers played a role in the

production of that damage. Although mammalian tissues contain PRE, expression of the phr gene in cells in culture has stringent requirements. We have thus developed a method of polyethylene glycol insertion of E. coli enzyme into mammalian cells. This should allow extension of the 'PR test' to cells with low intrinsic PRE levels (e.g. xeroderma cells, rodent cells) or to conditions unfavorable for PRE production.

We are using an alkaline agarose gel technique for measuring dimers in (non-radioactive) human skin. We shall examine repair by excision and photoreactivation in human skin exposed to light from different sources; we shall obtain action spectra for dimer production in leukocytes, and in mouse and human skin.

We have developed a system for UV-transformation to anchorage-independent growth of cultured human cells. We shall characterize the transformation process and the transformants, and determine the relation of this transformation procedure to solar oncogenesis in man.

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228. VIRUSES AS PROBES IN THE BIOCHEMISTRY OF MUTAGENESIS IN MAMMALIAN CELLS - MUTAGENIC DNA REPAIR IN RADIATION-ENHANCED REACTIVATED ADENOVIRUS

McLennan, A. G., Piperakis, S., University of Liverpool, Dept. of Biochemistry, P.O. Box 147, Liverpool L69 3BX, England, United Kingdom

OBJECTIVE: The objective of the project is to characterize error-prone DNA repair systems in human cells to clarify their proposed roles in carcinogenesis.

APPROACH: The system used for study is the radiation-enhanced reactivation of human adenovirus 2. Cells (HeLa and skin fibroblasts -both normal and XP) are treated with low doses of UV light and infected with adenovirus which has been irradiated with such doses of UV as will dimerize a significant number of the available adjacent thymine bases in its genome. Surviving viral plaques are cultured, DNA prepared, and the restriction patterns of these DNAs examined on polyacrylamide-agarose gels following digestion with the restriction enzymes EcoRI, HindIII and HpaI. The recognition sites of these enzymes (which number 25 in all in adenovirus 2) possess adjacent thymine bases. Misrepair of a dimer within such a site results in an altered restriction pattern of the progeny viral DNA. The frequency of such restriction site deletions is compared to that calculated for error-free host cell reactivated virus and indicates the level of induction of error-prone repair by UV light in the various cell types examined.

The exact nature and extent of the misrepaired sequences can be determined by further restriction digestion and sequencing of the regions surrounding the wild-type and the altered sites.

229. ENHANCED SURVIVAL OF UV-IRRADIATED HERPES SIMPLEX VIRUS IN UV OR CARCINOGEN PRETREATED HUMAN SKIN CELLS (ABBREV)

Coppey, J. M., Menezes, S., Fondation Curie, Dept. of Radiobiology, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 5, France

OBJECTIVE: The survival of UV-HSV is strongly enhanced in CV-1 monkey kidney cells when they are pretreated with small doses of UV or carcinogens (Lytle, Coppey and Taylor, Nature (1978) 272, 60). This enhancement seems to be related to an error-prone repair process (SOS repair) since the reactivated virus is highly mutated (Das Gupta and Summers, PNAS (1978) 75, 2378). Our purpose is to analyse this phenomenon in human skin fibroblasts in order to obtain further information on the extent of SOS repair in relation to the origin of the host cells.

APPROACH: Subcultivation of skin fibroblasts from a) normal donors, xeroderma pigmentosum (XP) heterozygotes (parents of XP), normal brothers of XP children; b) classical XP cases, XP variants, xerodermodysplasia verruciformis, Mibelli's porokeratosis, diffused naevomatosis, stuccokeratosis: all cases of the group b) present skin cancers (in the area exposed to sunlight) with a hereditary factor.

UV exposure or treatment with carcinogen (DMBA, EMS) of the growing cultures 0 to 60 h before infection with UV-HSV. Measurements of one cycle viral yields (methods: see Coppey et al., Bull. Cancer (1978), 65, 23).

3. EFFECT OF RADIATION ON DNA REPAIR IN NON-HUMAN CELLS

230. REPAIR OF EXPRESSION OF DNA DAMAGE INDUCED BY CHEMICAL AND PHYSICAL CARCINOGENESIS

Cerutti, P. A., Wang, T., Kaneko, M., Hirschi, M., Inst. Suisse de Recherches Experimentales sur le Cancer, Dept. of Carcinogenesis, Chemin des Boveresses, rue Bugnon 21, CH1066 Epalinges, Lausanne, Switzerland

OBJECTIVE: Study of the formation, repair and biological expression of DNA damage which is induced in mammalian cells by the important human carcinogens benzo(a)pyrene, aflatoxin B1 and ultraviolet light.

APPROACH: a) The capacity of actively metabolizing cultured human and rodent cells to excise covalent carcinogen-DNA adducts induced by the procarcinogens benzo(a)pyrene and aflatoxin B1 and the ultimate carcinogen N-acetoxy-acetylaminofluorene is being assessed. Cells are prelabeled with 14C-thymidine and treated with 3H-labeled carcinogen, and the DNA-adduct level is determined in enzymatic hydrolysates by chromatographic methods. In the case of mouse embryo cells C3H/10T1/2CL8 lesion excision is measured in confluent cultures and related to transformation frequencies following release from confluency by replating at low cell density. b) The formation of DNA lesions via the intermediacy of active oxygen species by indirect action of chemical DNA damaging agents such as benzo(a)pyrene, 4-nitroquinoline-N-oxide and ascorbic acid/Cu ion is measured in actively metabolizing human cells. DNA strand breaks and thymine damage are determined. c) The formation and repair of DNA damage by near-ultraviolet light in skin fibroblasts of patients with hereditary diseases with increased sensitivity to sun light are being investigated by the alkaline elution procedure.

231. REPAIR OF RADIATION-INDUCED LESIONS IN DNA Smith, K. C., Wang, T. V., Sargentini, N. J., Stanford University, School of Medicine, Dept. of Radiology, 300 Pasteur Dr., Stanford, California, 94305, United States of America

OBJECTIVES: (1) We plan to confirm our preliminary work with E. coli K-12 that, with UV radiation, back mutations occur by a one-hit process, and suppressor mutations occur by a two-hit process. (2) As controls for previous mutation studies, we observed that the different DNA repair deficient strains showed different levels of spontaneous mutagenesis in the same relative manner that these repair genes controlled UV radiation mutagenesis. To examine this phenomenon in more detail, we have studied a reversion system, but now need to study some forward mutations. We will also test various growth conditions for their possible effects on the level of spontaneous mutagenesis. (3) The recB, lexA and uvrD genes appear to act additively for survival after UV irradiation, while the recF mutation acts synergistically with these mutations. We have a new mutation (radA) that sensitizes a uvrB strain. We will test if this gene functions in the recF or the recB, uvrD, lexA pathways of postreplication repair. We will also study the effect of the radA and recF genes on the UV radiation-induction of resistance to UV radiation.

232. THE ROLE OF AN INDUCIBLE ERROR-PRONE DNA REPAIR PATHWAY IN MUTAGENESIS AND CARCINOGENESIS

Setlow, R. B., Rosenstein, B. S., Brookhaven National Lab., Dept. of Biology, Upton, New York, 11973, United States of America

The objective of this research project is to investigate the existence and properties of an inducible error-prone DNA repair pathway in animal cells that may play a role in mutagenesis and carcinogenesis. To accomplish this objective the effects of UV light and various chemical carcinogens will be examined using as parameters the rate of postreplication repair and frequency of mutant production. A haploid cultured cell line, ICR 2A, derived from the frog *Rana pipiens* will be used in these studies.

The specific aims of the proposal are: 1) To determine at what point in the cell cycle after UV-irradiation in the G1 phase: a) mutant production is no longer photoreversible, and b) the enhancement in postreplication repair after split dose treatment is no longer photoreversible. 2) To determine the relative abilities of wild type strains as compared to strains deficient in postreplication repair to a) undergo postreplication repair and mutant production, and b) exhibit the enhancement in postreplication repair after split dose treatment.

Through these studies the signal that triggers the error-prone pathway and the mechanism of this DNA repair process will be investigated. In current work with the ICR 2A cell line the effects of UV light and chemical carcinogens on mutant production and DNA repair systems are being studied.

233. DNA REPAIR AND ITS RELATIONSHIP TO CANCER

Friedberg, E. C., Bonura, T., Stanford University, School of Medicine, Dept. of Pathology, 300 Pasteur Dr., Stanford, California, 94305, United States of America

Studies on the enzymology and molecular mechanisms of the excision of damaged bases from DNA are in progress. These studies involve the isolation, purification and characterization of DNA glycosylases as well as specific endonucleases involved in the repair of damaged, inappropriate and missing bases from DNA, using extracts of prokaryotes, as well as extracts of yeast and mammalian cells.

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234. RADIATION REPAIR IN DROSOPHILA MELANOGASTER

Wallace, S. S., Margulies, L., New York Medical College, Westchester Campus, School of Medicine, Dept. of Microbiology, Valhalla, New York, 10595, United States of America

Drosophila melanogaster, a genetically well-characterized organism, is especially suitable for the elucidation of the biochemical basis of DNA repair mechanisms in eukaryotes. Since the various modes of DNA repair are intimately related to DNA replication, mutagenesis, and recombination; the enzymes involved in the correction of DNA damages may be instrumental in the carcinogenic process. The proposed research is designed to screen for specific repair endonuclease activities in wild-type *Drosophila* and in various known repair-deficient mutants. In order to assay for such damage-specific endonucleases, DNA substrates will be prepared by treatment with ultraviolet light, X-rays and alkylating agents.

235. REPAIR OF DNA TREATED WITH CHEMICAL CARCINOGENS AND GAMMA IRRADIATION

Goldthwait, D. A., Case Western Reserve University, Graduate School, Dept. of Biochemistry, 2040 Adelbert Rd., Cleveland, Ohio, 44106, United States of America

The isolation, purification and characterization of DNA repair enzymes in *E. coli* is a major objective of this laboratory. Further work is required to clarify previous studies on endonucleases II and VI of *E. coli*. An examination of N-glycosylases specific for alkylated bases is planned, as well as a search for exchange reactions. Further work on beta-propiolactone derivatives of DNA, their structure, and repair will be done. A second major research effort involves a search for N-glycosylases in mammalian tissue. Current studies involve rat liver and placenta. With a repair enzyme which recognizes alkylated DNA, a study of the removal of alkylated bases from chromatin and nucleosomes will be started. Finally, a cell culture system will be identified in which a careful kinetic study of repair of DNA alkylated by MNU can be done with the possibility of eventually integrating in vitro and in vivo findings.

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Effect on In Vivo Sensitivity to Methyl-Methane Sulfonate, *Biochemistry* 16: 5625 (1977).

236. MODIFICATION OF X-RAY INDUCED DAMAGES IN PHAGE T4

Wallace, S. S., DeVries, J., Jacobs, S. L., New York Medical College, Westchester Campus, School of Medicine, Dept. of Microbiology, Valhalla, New York, 10595, United States of America

The enzymes responsible for repairing DNA damage also function in several biologically significant pathways such as DNA replication, genetic recombination, and mutation fixation; and are thus directly implicated in the oncogenic process. Not only is little known about the repair of ionizing radiation-induced damages in DNA, but the chemical nature of these damages remains to be elucidated. The proposed research would be undertaken in an effort to define the molecular mechanisms involved in the repair to X-ray-induced damages in DNA using T4 bacteriophage and its *Escherichia coli* host as model systems. To this end, the DNA synthetic patterns of the recombinational repair deficient T4 x and y mutants have been examined, and the existence of aberrant DNA replicative intermediates established. An attempt will be made to isolate the products of the x and y genes by a complementation assay. Further, the *E. coli* X-ray endonuclease will be purified, and its properties delineated. Lastly, irradiated phage DNA will be repaired in vitro by purified enzymes of known specificities, and assayed for biological activity by transformation or transfection.

237. CELLULAR TRANSFORMATION AND SOS INDUCTION BY DNA INITIATED IN VITRO

Weinstein, I. B., Kirschmeier, P. T., Columbia University, College of Physicians & Surgeons, Division of Environmental Sciences, 630 W. 168th St., New York, New York, 10032, United States of America

I plan to examine the structural requirements and specificity of initiated DNA and the role of SOS functions in initiation and promotion of cellular transformation. DNA isolated from mouse and hamster cells, herpes and SV40 viruses, *Escherichia coli*, and phi X174 will be treated in vitro with benzo(a)pyrene, ultraviolet light or other initiating agents. Mouse and hamster cell lines will be transfected by initiated DNA. Transfected recipient populations will be scored for transformation, mutation induction or proviral induction to determine the transformation efficiency of homologous and heterologous DNA, the SOS induction efficiency of homologous and heterologous DNA and the damaged DNA structures that are the most potent transforming agents. In some experiments tumor promoter will be added to transfected cells because it should potentiate the transformation, mutation or proviral induction by initiated DNA and/or because transfected cells may require promotion for transformation, mutation or viral induction.

238. COMPLEMENTATION OF CELLULAR RADIATION REPAIR PATHWAYS

Elkind, M. M., University of Chicago, Pritzker School of Medicine, Dept. of Radiology, 5801 S. Ellis Ave., Chicago, Illinois, 60637, United States of America

Radiation sensitive V79 Chinese hamster cells, selected after a ouabain challenge, were found to be sensitive to the usual freeze (liquid N₂)/thaw cycle employed for storing mammalian cells. In contrast to parental cells with which recoveries of 75-100 percent are obtained, fewer than one percent of many sensitive clonal isolates were recovered as viable, colony forming cells.

Treatment after X-radiation with medium containing 90% D2O led to the expression of potentially lethal damage. Similarities were observed with postirradiation treatment with anisotonic buffer in respect to both cell killing and the repair of DNA damage (single-strand breaks expressible at high pH).

239. THE ROLE OF INDUCED PROTEIN SYNTHESIS IN CHEMICAL AND UV MUTAGENESIS

Clark, A. J., Uhlin, B., Volkert, M., Farrell, E., University of California, Berkeley Campus, School of Letters & Science, Dept. of Molecular Biology, Berkeley, California, 94720, United States of America

The overall objective of this research is to examine the importance of induced protein synthesis in UV and chemical

mutagenesis in *E. coli*. We have evidence that indicates there are two pathways of UV mutagenesis both of which are inducible. We think that the minor one, which results in 15-20% of UV induced mutations in phage and possibly bacteria, is one called SOS or error-prone repair. Part of our work will be to confirm the dependence of this pathway (transdimer DNA synthesis) *in vitro* and to search for mutants in which this pathway is more effective at producing mutations. Another part of our work involves testing for the dependence of *recF* of chemical mutagenesis. We will focus on three chemicals in particular: an alkylating agent (N-methyl-N'-nitro-N-nitrosoguanidine) which produces base substitution mutations, an adduct former (nitrazole) which produces frameshift mutations and a crosslinking agent (mitomycin C). The objective is to determine whether mutagenesis by these agents occurs by inducing components of some new pathway. To the extent that these results can be used to improve bacterial tests for mutagens and carcinogens strains will be constructed and tested.

240. CELLULAR HEREDITY IN HAPLOID CULTURES OF SOMATIC CELLS

Freed, J. J., Inst. for Cancer Research, 7701 Burholme Ave., Philadelphia, Pennsylvania, 19111, United States of America

Our objective is to study how the mode of repair of 254 nm ultraviolet induced DNA damage influences the probability of mutation in cultured somatic cells. DNA repair in bacteria can proceed by error-prone or error-free pathways and mutagenesis is enhanced by the former. We wish to see if this is true of vertebrate cells and to use the relationship to test whether 'cell culture variants' arise from effects on DNA. Using haploid cultures of frog cells (ICR 2A, haploid *Rana pipiens* embryo) we will investigate how the UV-induced yield of 6-thioguanine- and ouabain-resistant colonies is influenced by mode of repair, comparing error-free photoreversal (light) and error-prone postreplication repair (dark). We will also seek, in split-dose experiments, effects on cell survival and induced mutagenesis indicative of an error-prone induced repair pathway of the SOS type.

RESULTS: ICR 2A cells have been shown to be excision-repair defective but to have high capacity for photoreversal of pyrimidine dimers, and consequent ability to photoreactivate survival. In preliminary studies the frequency of 6-thioguanine- and ouabain-resistant colonies among survivors was elevated by 254 nm irradiation if recovery proceeded in the dark but reduced if photoreversal (white-light illumination) was allowed to occur.

241. EFFECT OF ULTRAVIOLET LIGHT ON CELLULAR PROCESSES

Tessman, I., Fassler, J. S., Purdue University, School of Science, Dept. of Biological Sciences, Executive Bldg., West Lafayette, Indiana, 47907, United States of America

The object is to understand the effects of ultraviolet radiation (UV) on cellular processes, the repair of damages caused by the radiation, and the mutational consequences on the repair processes. A recently discovered effect of UV is the suppression of polarity, which is a premature termination of transcription. A theory will be tested that the UV depletes the activity of the transcription termination factor rho. Polarity will be studied in two genetic systems, the genes of bacteriophages phi X174 and G4, and the genes of the *gal* operon. The aims are to explain the function of the rho protein in the repair of cell damage and how UV prevents transcription termination. UV can also cause termination of transcription and that property will be used to provide a functional test for promoters. The aims are to determine if the use of distant promoters rather than proximal ones in a general strategy of viruses and to determine what signals are involved in specifying the translatable part of a messenger RNA. UV induces a repair process that is highly mutagenic. A mechanism has been proposed to explain the mutagenic effect and the theory will be tested by direct determination of the base changes produced by the mutations, and that will be done by DNA sequencing.

242. RADIATION INDUCED MUTAGENESIS AND CARCINOGENESIS

Evans, H. H., Case Western Reserve University, School of Medicine, Dept. of Radiology, 2109 Adelbert Rd., Cleveland, Ohio, 44106, United States of America

The objective of this research is the investigation of the role of DNA repair in radiation-induced mutagenesis and transformation in a mammalian cell line. The effects of stationary holding recovery on the frequencies of these processes is currently under investigation. The characteristics of DNA repair in the cells will be studied by measuring error-free and error prone reactivation of irradiated virus by the host cells under various conditions. We also hope to isolate repair-defective strains following mutagenesis of the cells with ethylmethane sulfonate (EMS) and enrichment using a host-cell viral reactivation suicide technique. Changes in the frequencies of carcinogenesis and mutagenesis in the wild-type cells are compared to the repair-deficient strains will then be correlated to deficiencies in specific repair processes.

243. MOLECULAR BASIS OF RADIATION LETHALITY

Smith, K. C., Diver, W. P., Sargentini, N. J., Stanford University, School of Medicine, Dept. of Radiology, 300 Pasteur Dr., Stanford, California, 94305, United States of America

To map, purify and study the repair deficiencies after ionizing irradiation of a new class of mutants of *E. coli* that we have isolated that are largely UV resistant but are ionizing radiation sensitive. The strains currently being purified are called *irrB* and *irrC*, and map near *leu*, and *dnaA*, respectively. Strains 41, 205, 211 and 232 map near but do not cotransduce with his. Additional strains are being developed using transposon insertion mutagenesis. The *irrA* strain appears to modify rich growth medium-induced resistance to radiation damage, a process controlled by the *recA*, *recB*, and *lexA* genes.

244. INTERACTION OF ULTRAVIOLET LIGHT AND CHEMICAL CARCINOGENESIS

Starkey, J., Washington State University, School of Veterinary Medicine, Dept. of Veterinary Microbiology & Pathology, Pullman, Washington, 99163, United States of America

OBJECTIVE: Determine the effect of the interaction of UV irradiation and other environmental carcinogens on DNA conformation and DNA repair mechanisms.

APPROACH: Treat mammalian cells in culture with various polycyclic hydrocarbon carcinogens and then with UV irradiation. Determine effect on DNA repair response, DNA conformation, and stability of DNA and DNA-protein complexes.

245. RADIATION AND CHEMICAL DAMAGE

Sellow, R. B., U.S. Dept. of Energy, Brookhaven National Lab., Dept. of Biology, Upton, New York, 11973, United States of America

The responses of cells to the deleterious agents in the environment that arise from nuclear, fossil and synthetic fuel technologies are governed by the fact that DNA is not only the most vulnerable but also the most important cellular target. Thus cellular responses depend on the radiation and chemically induced changes in DNA and how cells cope with these changes. Our goal is to understand the repair systems in a number of different animals, how they mitigate deleterious effects, and if such systems are inhibited by pollutants from fuel technologies. We will analyze, at the molecular level, the characteristics of the repair steps that take place both before and after DNA replication in bacteria and mammalian cells. We will attempt to correlate biological, enzymological, chemical and physical data on bacterial and various types of mammalian cells so as to identify quantitatively the changes in DNA that have serious biological consequences, and determine how physical and chemical agents affect the repair systems themselves.

246. EFFECTS OF FAST NEUTRONS ON CULTURED MAMMALIAN CELLS

Moss, A. J., Nagle, W. A., Baker, M. L., Prior, R. M., University of Arkansas, Medical Sciences Campus, School of Medicine, Dept. of Medicine, 4301 W. Markham St., Little Rock, Arkansas, 72201, United States of America

This study is concerned with the cellular and molecular effects of fast neutrons on cultured mammalian cells. The experiments involve the production of damage in intracellular DNA, which is later measured, as well as an assessment of the cells' ability to repair the damage. The results with fast neutrons will be contrasted with those obtained with 250 kVp X-rays. The data will provide estimates of the RBE and OER for fast

neutrons using both molecular and cellular end points. The influence of various conditions such as pH, hypoxia and available energy (ATP) on the rate and extent of DNA repair will provide information on the basic mechanisms of fast neutron effects on mammalian cells.

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247. CELLULAR RECOVERY MECHANISMS, DNA REPAIR AND RADIATION SENSITIZATION

Howard Flanders, P., Yale University, School of Medicine, Dept. of Therapeutic Radiology, 333 Cedar St., New Haven, Connecticut, 06510, United States of America

This is part of a broader project. A summary of this subproject is not available.

248. RADIATION AND CHEMICAL IN VITRO MALIGNANT TRANSFORMATION

Kennedy, A. R., Little, J. B., Aebersold, P. M., Eisenstadt, E., Harvard University, Boston Campus, School of Public Health, Dept. of Physiology, 55 Shattuck St., Boston, Massachusetts, 02115, United States of America

The basic objective of this research program is to gain specific information about the mechanisms of radiation carcinogenesis using in vitro assay system. Previous results indicate that X-ray transformation in vitro can be thought of as involving at least two steps: 1) the fixation of the transformed state (presumably irreversible DNA damage) and 2) its subsequent expression in terms of morphologically altered cells. We will investigate in detail the conditions necessary for fixation and ultimate expression of the cellular damage induced by ultraviolet light and ionizing radiation which results in malignant transformation of cells. In particular, we plan to study the role that DNA repair processes play in radiation-induced transformation and the interactions between radiation and chemical agents in malignant transformation. At present, little information is available on the interactions between radiation and other agents from in vivo carcinogenesis studies or in vitro transformation work, although human populations are continually being exposed to a variety of carcinogenic agents as well as to other agents that can influence carcinogenesis.

The cell system we will use is an established mouse embryo cell line which has been used for several years in this laboratory for radiation transformation experiments and is particularly appropriate for studying interactions between radiation and chemical agents. The specific chemical agents we wish to study have known interactive effects on carcinogenesis in vivo; studies in an in vitro system in which cell proliferation and other environmental conditions can be precisely controlled should yield further information about the mechanisms of carcinogenesis.

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249. RADIATION BIOLOGY CORE

Fischer, J. J., Yale University, School of Medicine, Dept. of Therapeutic Radiology, 333 Cedar St., New Haven, Connecticut, 06510, United States of America

This is part of a broader project. A summary of this subproject is not available.

4. OTHER EFFECTS OF RADIATION ON THE GENETIC APPARATUS

250. ESR STUDY ON HYDROCARBON RADICALS AND CARCINOGENESIS

Ts'o, P. O., Lesko, S. A., Drocourt, J. L., Greene, J. J., Johns Hopkins University, School of Hygiene & Public Health, Dept.

of Biochemistry, 615 N. Wolfe St., Baltimore, Maryland, 21205, United States of America

The mechanism by which ionizing radiation and reduced oxygen species damage DNA/genetic apparatus in vitro and in mammalian cells will be examined. DNA interstrand crosslinking and DNA-protein crosslinking induced in isolated chromatin by H₂O₂/Fe ions, H₂O₂ plus near UV light and ionizing radiation will be investigated. An attempt will be made to quantitatively compare DNA strand scission and DNA interstrand crosslinking in homogenous T7 DNA. The technique of high resolution mass spectrometry will be used to analyze for base modification induced in DNA. It appears that modified bases can be released from DNA more easily than unmodified bases. Mammalian cells will be examined for the presence of DNA strand scission, DNA-protein crosslinks, DNA interstrand crosslinking and modified bases after treatment with H₂O₂ plus near UV light.

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251. MUTAGENIC SPECIFICITY OF ENVIRONMENTAL CARCINOGENS

Eisenstadt, E., Haseltine, W. A., Brash, D., Harvard University, Boston Campus, School of Public Health, Dept. of Microbiology, 55 Shattuck St., Boston, Massachusetts, 02115, United States of America

The basic objective of this research program is to develop specific information about the spectrum of mutations that can be induced by environmental carcinogens. We propose to study, at the nucleotide level, the DNA alterations that are generated by carcinogens in the *lacI* gene of *Escherichia coli*. We will determine the mutagenic spectrum induced by ultraviolet light and cyclopenta(cd)pyrene in bacteria with different DNA repair capabilities. The information we expect to obtain from these studies includes: 1) the full range of mutational events that arise from damage to DNA by selected environmental carcinogens; 2) the influence of certain genes governing the enzymatic repair of DNA lesions on the mutagenic spectrum of carcinogens; 3) the effect of primary structure (sequence) on the conversion of DNA lesions to mutations.

To develop this information, we shall induce and map mutations in the *lacI* gene carried on an F' factor. The mutation will then be crossed onto a plasmid whose copy number can be amplified. The isolated plasmid will be cut with a restriction enzyme to remove the region containing the *lacI* gene. The 5' ends of this fragment will be labelled with 32p and the labelled DNA will be cut with a set of restriction enzymes to generate fragments that are suitable for sequencing by the Maxam-Gilbert method.

252. GENETIC STUDIES OF CELLULAR RESISTANCE TO PHYSICAL AND CHEMICAL CYTOTOXIC AGENTS

Boyle, J. M., Durrant, L. G., Paterson Lab., Dept. of Experimental Chemotherapy, 550 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom

OBJECTIVE: To investigate dominance and complementation of genetic factors affecting cell survival and mutagenicity in cell lines with differential sensitivity to cytotoxic agents.

APPROACH: 1. Pairs of rodent tumor cell lines have been chosen which appear to show differential sensitivity to a specific DNA damaging physical (UV or X-rays) or alkylating (e.g., MDMS) agent. 2. The patterns of cross-sensitivities to other cytotoxic agents are being determined. 3. Mutation induction is being measured for thioguanine and ouabain resistances, and will provide marked cell lines for fusion studies. 4. Cell survival, mutagenicity and karyotypes of hybrid cells will be compared with those of parent cells. 5. Where complementation patterns are found to give clearly distinguishable classes, biochemical characterization of DNA repair may be possible. 6. Where practicable, human and rodent fusions will be made which may provide material for future mapping studies.

253. INTERSPECIFIC COMPARISON OF SENSITIVITY TO CHROMOSOME ABERRATION PRODUCTION BY X-RAYS

Fischer, P., Universität Wien, Inst. fuer Krebsforschung, Dept. of Cytogenetics, Borschkegasse 8A, A1090 Vienna, Austria

An extrapolation of data from animal experiments can only be of value when it has been proved that results in both species are comparable.

We have previously investigated the incidence of chromosome aberrations in man in first post-irradiation divisions using the incorporation of BrdU as a marker. The incidence of first division aberrations in marmosets will now be tested and compared with the human data.

254. TRANSPOSABLE ELEMENTS IN DROSOPHILA

Rubin, G. M., Lewis, R., Brown, P., Sidney Farber Cancer Center Inc., Dept. of Basic Sciences, 44 Binney St., Boston, Massachusetts, 02115, United States of America

The general aim of the proposed study is to determine aspects of the structure and function of certain transposable genetic elements in *Drosophila melanogaster*. The properties of three transposable elements, 412, copia, and 297, will be investigated in detail using recombinant DNA techniques. The mechanism(s) and rate(s) of transposition of these elements in *Drosophila* cell culture lines will be investigated. The effect of various mutations, chemical agents (mutagens, tumor promoters, etc.), and irradiation (u.v. and x-ray) on these rates will be determined. The nature and developmental specificity of the gene products of these transposable elements will be studied.

255. BIOLOGICAL AND PHYSICAL ACTION OF RADIATION ON DNA

Achey, P. M., State University System of Florida, University of Florida, School of Agriculture, Dept. of Microbiology, Gainesville, Florida, 32601, United States of America

Throughout the project, the single-strand and double-strand forms of bacteriophage phi X174 DNA will be used as a model system to study the molecular action of ultraviolet (UV) and ionizing radiation on DNA. There are two major objectives of this proposal; first, to study the influence of the molecular environment of DNA (both chemical and enzymatic) on its radiosensitivity, and the types of damage produced by the radiation; and, second, to determine which types of radiation damage in DNA are biologically important with regard to mutagenesis and lethality. Ionizing radiation produces strand breaks and base damage in DNA. The chemistry of the strand breaks and the types of base damage varies in response to changes in the chemical environment of the DNA at the time of irradiation. Experiments will be designed to study the response of damage produced under different radiation conditions to various repair systems, especially the induced repair system. Several agents are known which can introduce specific types of base damage, including alkylating agents (e.g., methyl methanesulfonate) and osmium tetroxide (which only reacts with single-strand DNA). Exposure of DNA to near UV radiation gives a different spectrum of base damage than exposure to far UV. Mild heat treatment of alkylated DNA, or more extensive heat treatment of normal DNA, leads to depurination. Biological killing and mutagenesis in DNA damages by these types of agents will be studied, for the purpose of determining the biological consequences resulting from base damages of various types.

The broad objective of this project is to determine the mechanism(s) of killing and mutagenesis by radiation with particular emphasis on the biological significance of various types of radiation damage and the modification of the damage by cellular repair systems.

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256. IN VITRO STUDY OF MAMMALIAN CELLS DAMAGED BY TRITIUM

Burki, H. J., University of California, Berkeley Campus, Lawrence Berkeley Lab., Berkeley, California, 94720, United States of America

Mouse leukemic cells (L5178Y), human cells (HeLa), Chang's liver (WI38), and several types of Chinese hamster cells from different tissue sources (V79 lung, M3-1 bone marrow, CHO ovary), will be labeled with precursors of DNA containing tritium, ¹⁴C, ¹²⁵I, Br, or tritiated precursors of protein and RNA. After being synchronized by various methods, these cells will then be cooled to 4 degrees C or frozen to minus 196 degrees C to accumulate damage due to tritium and other isotope disintegration. After suitable time intervals, the cells will be thawed out and the reproductive ability, chromosome damage, and induced mutations will be determined. The efficiency of cellular inactivation and the induction of genetic damage will be compared for damage accumulated in various subcellular regions.

These experiments are designed to further explore regions within the mammalian cell nucleus where isotopic decay appears to be most critical in the induction of reproductive death, chromosome damage, and mutations. We are particularly interested in the DNA which replicates late in the cell cycle, including some of the sex chromosomes and DNA near the nucleus membrane, as well as tritium damage in satellite DNA. Two other experimental parameters, which appear to be quite important, the time in the cycle of the accumulation of damage, and the quality and the reparability of the damage that is formed, will also be studied.

The results of these studies may provide information of two general types: (1) information on the basic mechanisms which lead to the inactivation of proliferative ability and mutagenesis of mammalian cells and the induction of chromosome aberrations, and (2) quantitative estimates of the relative hazards to the population from environmental tritium released to the biosphere as a by-product of fission and fusion power production.

257. TRITIUM DAMAGE IN MAMMALIAN CELLS

Burki, H. J., University of California, Berkeley Campus, Lawrence Berkeley Lab., Berkeley, California, 94720, United States of America

One major objective of these studies is to gain information on the mechanisms which lead to inactivation or the mutagenesis of several types of mammalian cells in vitro. The other major objective is to provide a quantitative framework for evaluating the effects of tritium disintegrations and related types of damaging agents in different parts of the mammalian nucleus, including hot spots for killing and mutagenesis. Using agents like ³H, ¹⁴C, ¹²⁵I, Br, and I incorporated into the DNA of synchronized mammalian cells, the resulting effects on mutations and other experimental endpoints will be related to the time in the life cycle when the effects are important and the position of the DNA where the damage occurs. These results will be compared to results obtained from exposure to random damaging mutagenic agents. Certain genetic studies not yet possible in mammalian cells will be performed using diploid yeast. This information will be useful in constructing conservative estimates of world health effects due to tritium by products associated with fission and proposed fusion power generation.

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258. EFFECTS OF X-RAYS ON NORMAL AND MALIGNANT CELLS

Tolmach, L. J., Beetham, K. L., Balachandran, R., Washington University, School of Medicine, Dept. of Radiology, 660 S. Euclid Ave., St. Louis, Missouri, 63110, United States of America

X-ray-induced perturbations of mammalian cell growth and proliferation will be studied, with emphasis on DNA synthesis and cell killing. The relation of these two manifestations of radiation insult will be examined in terms of a general model involving potentially lethal damage and its repair. The effects of different concentrations of caffeine on the survival of irradiated cells and on the suppression of radiation-induced inhibition of cell progression will be examined. The relation between the potentially lethal damage expressed by treatment of irradiated cells with two different agents or conditions will be studied.

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259. ANALYSIS OF X-RAY AND HEAT-INDUCED MUTAGENESIS

Hopwood, L. E., Medical College of Wisconsin, School of Medicine, Dept. of Radiology, 561 N. 15th St., Milwaukee, Wisconsin, 53233, United States of America

The principal objective of this research is to obtain quantitative data on dose-response relationships for the induction of mutation in an in vitro mammalian cell system. CHO and HeLa cells will be selected for their resistance to 8-azaguanine, a toxic analogue which selects for a mutation in the gene for hypoxanthineguanine phosphoribosyl transferase; and to ouabain, which in its binding to ATPase is toxic to wild-type cells. Factors which influence the quantitative nature of the mutation system, such as drug concentration, cell density and expression time, will be carefully controlled to obtain accurate responses. The mutation induction by x-rays will be determined for different stages of the cell cycle, various dose-rates and fractionations. The possible influence of hyperthermia on mutation production will be investigated. Various agents and treatments will be used to measure the repair of the mutation lesions in comparison with that of the lethal lesion. An analysis of mutants selected under several conditions will be aimed at determining the nature of the mutation lesion.

260. ORIGIN AND DEVELOPMENT OF CANCER STUDIED WITH CELL MARKERS

Fialkow, P. J., Reddy, A. L., Collins, S. J., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Medical Service, 4435 Beacon Ave. S., Seattle, Washington, 98108, United States of America

The objective of this project is to use chromosome markers and a system of naturally occurring isoenzyme-marked genetic mosaicism based upon X-chromosome inactivation to investigate in mice: 1. The early steps in oncogenesis with special reference to determining at which stages chromosomal abnormalities and clonal proliferation occur. 2. The nature of the tumorigenic changes induced by chemicals, radiation and viruses. 3. The influence of host mechanisms on the frequency with which transformed cells occur and evolve into malignancies. Thus far our efforts have been directed to breeding feral mice with the X-linked isoenzyme marker (phosphoglycerate kinase type 1a) to inbred strains. We are at the following backcross generations: BALB/c, F8; AKR, F6; NZB, F4; C3H, F4, and RFM, F1. Experimental findings to date include the following: 1. Fibrosarcomas induced by the carcinogen 3-methylcholanthrene dissolved in olive oil have a multicellular origin, suggesting that the tumorigenic event induced by methylcholanthrene is not as rare as had been assumed previously. 2. If the carcinogen is dissolved in benzene, the resultant malignancies have a multicellular origin. 3. A specific chromosomal abnormality, trisomy 15, was found in two B-lymphoid neoplasms suggesting that contrary to what had been believed previously, this abnormality is not specific to T-lymphoid neoplasms. It is hoped that determining the number of progenitor cells for tumors that arise in mice under defined circumstances and correlating the results with similar data in spontaneously arising tumors in man will provide indirect evidence about causative factors in human tumorigenesis.

261. THE MECHANISM OF CELL TRANSFORMATION

DiPaolo, J. A., Doniger, J., Evans, C. H., Popescu, N. C., Greiner, J. W., Laramendy, M. L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Somatic Cell Genetics Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

The conditions which are responsible for modulation in vitro transformation will be investigated further, with specific emphasis on the use of UV, X-rays, combination of carcinogens of different classes, the combination of in vivo - in vitro systems, and the effect of repair mechanisms on toxicity and trans-

formation. The relevance of chromosome damage as a result of initial carcinogen insult as well as to which cytogenetic alterations are responsible for or associated with malignancy is being determined using cells from a variety of species including human. Studies on the mechanisms of early events of carcinogen-cell interaction will be continued, as well as investigation of different proximate carcinogens and metabolic inhibitors. Special emphasis will be given to the requirements necessary to obtain neoplastic transformation of human fetal cells. The testing for the tumorigenicity of cells exposed to different agents for different time intervals will continue.

BIBLIOGRAPHIC REFERENCES: DiPaolo, J.A.: Book review of *Carcinogenesis, A Comprehensive Survey*, Vol. 5, *Modifiers of Chemical Carcinogenesis: An Approach to the Biochemical Mechanism and Cancer Prevention*. Thomas J. Slaga, (Ed.): Raven Press, 1980. Cell, (In Press). Doniger, J., and DiPaolo, J.A.: Excision and post-replication DNA repair capacities, enhanced transformation, and survival of Syrian hamster embryo cells irradiated by ultraviolet light. *Cancer Res.* 40: 582-587, 1980.

262. POTENTIAL HAZARDS OF PHOTOTHERAPY

Speck, W. T., Carr, H. S., Case Western Reserve University, School of Medicine, Dept. of Pediatrics, 2109 Adelbert Rd., Cleveland, Ohio, 44106, United States of America

Large numbers of normal newborn infants are systematically exposed to a new and potentially harmful environmental agent routinely utilized for the treatment of neonatal jaundice - high intensity illumination with visible light or phototherapy - often for extended periods of time. The proposed research project will enable us to further characterize the DNA-modifying activity of phototherapy for eukaryotic cells in culture by analyzing the physical chemical properties of DNA isolated from cells exposed to phototherapy in vitro. We will then determine if similar DNA-modification occurs in animals exposed to various phototherapy regimens and determine the action spectra for these genetic effects in order to facilitate the development of a phototherapy unit effective in the treatment of hypervilurimemia yet devoid of DNA-modifying activity.

BIBLIOGRAPHIC REFERENCES: Speck, W.T., Santella, R.M., Rosenkranz, H.S.: Alteration of human cellular DNA by neutral red in the presence of visible light. *Mutation Res.* 66:95, 1979.

263. RADIATION-CHEMICAL INDUCTION OF MUTAGENESIS

Richmond, R. C., Dartmouth College, Dartmouth Medical School, Dept. of Radiation Therapy/Medicine, P.O. Box 833, Hanover, New Hampshire, 03755, United States of America

OBJECTIVES: Radiation sensitization of cell killing and of mutagenesis by combined treatment of *S. typhimurium* TM677 with radiation and electron affinic radiation sensitizers and antitumor drugs is to be characterized. DNA damage by these combined treatments is to be assayed, e.g., by strand break analysis.

METHODS: Survival curve analysis following combined treatment of cells in aqueous suspension by radiation and various sensitizers/drugs is compared with mutagenesis caused by these same treatments. The effect of oxygen on these endpoints is examined by treating cells in N₂- and O₂-saturated suspensions. DNA damage is evaluated by agarose gel electrophoresis and thymine residue release studies.

RESULTS: To date, the TM677 mutational assay to 8-azaguanine resistance is characterized for the effects of methyl methanesulfonate and N-methyl-N'-nitro-N-nitrosoguanidine. These agents are increased ca. 2-fold in effectiveness when present with cells under conditions of shift-up in growth rate.

264. RADIATION INDUCED ALTERATIONS OF CHROMOSOMAL PROTEINS

Roti Roti, J. L., Wilson, C. F., Utah Higher Education System, University of Utah, School of Medicine, Dept. of Radiology, 1400 E. 2nd S., Salt Lake City, Utah, 84112, United States of America

The proposed research is designed to determine the role of chromatin function and structure in the radioresponse of mammalian cells. Regarding chromatin function, we hope to determine: 1) whether radiation induces any changes in the rate of addition or replacement (turnover) of chromosomal proteins,

and 2) whether any radiation-induced changes in the turnover of chromosomal proteins play a role in radiation-induced division delay. Regarding chromatin structure, the objectives are: 1) to determine whether the in situ arrangement of chromosomal proteins on the DNA molecule or changes therein affect the induction of DNA damage and its repair, and 2) to determine what role thermally induced structural changes in the protein-DNA interaction in chromatin play in heat induced killing. Chromatin isolated from cultured mammalian cells will be used to assay both radiation and thermal effects. The isolated chromatin will be fractionated by gel electrophoresis so that alterations of individual protein components may be examined. The particular class of DNA damage we will study is the 5,6-dihydroxydihydrothymine (T gamma)-type products. T gamma-type products are formed in DNA by OH radicals and are removed by an excision repair process. We will examine the formation and repair of T gamma-type products as a function of the cell's position in the cell cycle and as a function of the DNA-protein organization of chromatin.

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265. RADIATION-INDUCED MODIFICATIONS IN PROTEIN SYNTHESIS

Oleinick, N. L., Biaglow, J. E., Chiu, S., Case Western Reserve University, School of Medicine, Dept. of Radiology, 2109 Adelbert Rd., Cleveland, Ohio, 44106, United States of America

The objective of the research is to compare transcriptionally active and inactive chromatin with respect to (a) initial yields of ionizing radiation-induced DNA damage and (b) repair capacity. Since the specific model system will be ribosomal chromatin, which contains the DNA coding for ribosomal RNA (rRNA), the work will involve comparing the transcriptionally active ribosomal DNA characteristic of actively proliferating cells to the transcriptionally less active rDNA characteristic of quiescent (and generally more radioresistant) cells.

Rodent or human cell lines will be obtained during active exponential growth, during inhibition by nutrient or serum depletion, confluence, or drugs, or during stimulation with nutrients or insulin. The rate of ribosomal RNA synthesis and other metabolic parameters characteristic of proliferation rate (protein synthesis and phosphorylation, cyclic nucleotide levels) and radiation damage to and repair of rDNA will be determined. We have developed a system to analyze single-strand breaks, alkali-labile lesions, and enzyme-sensitive sites specifically in rDNA. The procedure involves releasing the rDNA from bulk nuclear DNA with a restriction endonuclease, treatment with a lesion-specific endonuclease, and sizing the rDNA fragments by agarose gel electrophoresis, then identifying rDNA by hybridization to rRNA. Furthermore, ribosomal chromatin from proliferating or plateau phase, irradiated or non-irradiated cultures will be tested for its accessibility to other nucleases reported to be specific for structural features of active or inactive chromatin.

Unlike the majority of nuclear DNA, the rRNA genes are a major class of DNA which is known to be differentially active in proliferating and plateau phase cells. Plateau phase cells have been considered a suitable two-dimensional model for the non-growing or slowly growing and generally more radioresistant subpopulations of tumors. Thus, the results will help to define some critical areas of metabolism affected when nutrients or hormones stimulate the proliferation of plateau phase cells and enhance their radiosensitivity.

266. BIOLOGICAL EFFECTS OF DNA-PROTEIN CROSSLINKS

Larcom, L. L., Dadds, E. G., Clemson University, College of Sciences, Dept. of Physics/Astronomy, Long Hall, Clemson, South Carolina, 29631, United States of America

The crosslinking of proteins to DNA has been proposed as a mechanism for aging and carcinogenesis, but the biological importance of these crosslinks has remained unclear. We have employed a transfection system to demonstrate that: 1) sensitivity of DNA to biological inactivation by ultraviolet radiation is

altered by the presence of proteins or amino acids, 2) this effect apparently does not require covalent crosslink formation, 3) UV sensitivity of DNA in buffer and in solutions of amino acids is a function of the cation concentration of the buffer. The mechanisms by which proteins alter UV sensitivity of DNA remain unclear.

This research will: 1) identify the protein-DNA interactions which contribute to UV sensitization of DNA by proteins, 2) determine whether proteins can enhance the production of non-crosslink lesions, attempt to identify cellular enzyme systems capable of detecting and repairing DNA-protein crosslinks, 4) evaluate the transfection system for its ability to elucidate interactions between specific initiators and promoters of carcinogenesis.

267. EFFECTS OF ULTRAVIOLET LIGHT ON EPIDERMAL POLYAMINE BIOSYNTHESIS AND DNA SYNTHESIS

Lowe, N. J., University of California, Los Angeles Campus, School of Medicine, Dept. of Medicine/Dermatology, 2025 Zonal Ave., Los Angeles, California, 90024, United States of America

The objective of this project is to further investigate the changing patterns of polyamine biosynthesis and DNA synthesis in the epidermis following different forms of ultraviolet light irradiation and the effects of different drugs and chemicals on these changes.

The approach is by examining the effects of ultraviolet light on hairless mouse epidermal polyamine and DNA biosynthesis in vivo. In addition, we are conducting studies using guinea pig skin because of the greater ease of observing cutaneous erythema in these animals.

PROGRESS: We have ascertained that predominantly UVB (290-320 nm) wavelengths induce epidermal polyamine biosynthesis. The induction of polyamine biosynthetic enzyme activity is modulated by chronic ultraviolet irradiation and also by antiinflammatory drugs and retinoids.

These studies may help to yield further information on mechanisms and potential modulation of ultraviolet light carcinogenesis.

BIBLIOGRAPHIC REFERENCES: Lowe, N.J. and Breeding, J.: Antiinflammatory Effects of Ultraviolet Light Induced Ornithine Decarboxylase and DNA Synthesis. *J.I.Derm.* 74:418-420, 1980. Lowe, N.J. and Breeding, J.: Evaluation of Sunscreen Protection by Measurement of Epidermal DNA Synthesis. *J.I. Derm.* 74:181-182, 1980.

268. TRANSFER MECHANISMS IN IRRADIATED BIOLOGICAL SYSTEMS

Box, H. C., Roswell Park Memorial Inst., 666 Elm St., Buffalo, New York, 14263, United States of America

This project is a study of radiation damage in nucleic acid constituents using a variety of experimental approaches including ESR, ENDOR and ¹³C NMR spectroscopy; also HPLC and mass spectrometry. Attention will be given to radiation effects in the carbohydrate and phosphate moieties of nucleic acids. Also of interest are the mediative effects of halogen substituted bases on the overall radiation damage process. For example, cocrystals of 5 bromouridine and adenosine, X irradiated at 4.20K, are being studied by ESR-ENDOR spectroscopy to determine how halogenation alters the primary radiation damage process.

BIBLIOGRAPHIC REFERENCES: Trapped Electrons in Irradiated Single Crystals of Polyhydroxy Compounds. Harold C. Box, Edwin E. Budzinski, Harold G. Freund and Williams R. Potter, *Journal of Chemical Physics* 70, 1320 (1979). Characterization of Trapped Electrons and Electron Traps in Single Crystals. Edwin E. Budzinski, Williams R. Potter, George Potienko and Harold C. Box, *Journal of Chemical Physics*, in press (1978).

269. LIGHT-INDUCED MODIFICATION OF DNA WITH COUMARYL COMPOUNDS

Song, P., Texas Tech University, School of Medicine, Dept. of Biochemistry, P.O. Box 4340, Lubbock, Texas, 79409, United States of America

Coumaryl compounds are synthesized naturally by plants and fungi and are common in our environment. Psoralens and aflatoxins, particular kinds of coumaryl compounds, contain a light-absorbing structure called coumarin, which is responsible

for the photochemical reactivity of these compounds. Psoralens are known to cause skin cancer in the presence of near ultraviolet light (UV), while aflatoxins (AF) are known to be a potent liver carcinogen as well as inducers of skin tumors in white mice. The toxic actions of these coumaryl compounds are apparently a result of their ability to modify the genetic material, deoxyribonucleic acid (DNA), which is present in every living cell. This award should elucidate the chemical nature of DNA modification by psoralens and AF. Specifically, the researchers will determine which component bases on DNA are photomodified by cancer-inducing psoralens and AF's and then evaluate the relative importance of DNA modification by psoralen and its derivatives. In addition, the utility of these newly-synthesized psoralen-labelled and AF-labelled DNA's as new research tools in molecular biology and cancer research will be ascertained.

270. EFFECTS OF SMALL DOSES OF IONIZING RADIATION

Rossi, H. H., Bird, R. P., Borek, C., Geard, C. R., Hall, E. J., Kliauga, P. J., Miller, R. C., Columbia University, College of Physicians & Surgeons, Dept. of Radiology, 630 W. 168th St., New York, New York, 10032, United States of America

The biological effects on mammalian cells of low doses of ionizing radiations will be investigated within the framework of a biophysical theory. A variety of endpoints will be observed including cell lethality, chromosome aberrations, sister chromatid exchanges and oncogenic transformations. The analysis of the data should permit extrapolations of the dependence of cellular effects to doses too low for experimental determination. Modifications of the dose response curves by environmental factors such as oxygen tension as well as chemical and pharmacological agents are an important related objective of the investigation. In addition to intrinsic scientific significance, the results of this research are of pragmatic value in radiation protection and radiotherapy.

A variety of radiations are to be applied including neutrons of a wide range of energies, x and gamma rays, alpha radiation and energetic ions up to Z equals 18. The higher organisms irradiated will be principally mammals but insects and plants are to be used in certain studies. The effects under investigation include carcinogenesis, cell transformation, chromosome aberrations and genetic impairment.

271. CHEMICAL AND RADIATION CARCINOGENESIS - BASIC MECHANISMS AND ESTABLISHMENT OF BIOMEDICAL RISK ASSESSMENT SYSTEMS

Ts'o, P. O., Johns Hopkins University, School of Hygiene & Public Health, Dept. of Biophysics, 615 N. Wolfe St., Baltimore, Maryland, 21205, United States of America

The objective is to investigate the basic mechanisms of chemical and radiation carcinogenesis so as to establish a biomedical risk assessment system for humans based on fundamental research. The biomedical risk assessment system depends on the construction of a universal biomedical risk equivalent system which, in turn, is based on 7 or 8 information matrices. These matrices are: (1) type of perturbation; (2) DNA damage; (3) alteration in organization and expression of genome; (4) somatic mutation; (5) neoplastic/malignant transformation; (6) correlation of in vitro and in vivo results; (7) hamster/human correlation; and (8) correlation between human individuals and human populations.

BIBLIOGRAPHIC REFERENCES: J.C. Barrett, T. Tsutsui, P.O.P. Ts'o, Neoplastic Transformation Induced by a Direct Perturbation of DNA, *Nature*, 274, p. 229, 20 July 1978; J.C. Barrett, N.E. Bias, P.O.P. Ts'o, Mammalian Cellular System for the Concomitant Study of Neoplastic Transformation and Somatic Mutation, *Mutation Res.*, 50, p. 121 (1978).

5. EFFECT OF RADIATION ON CELL MEMBRANES

272. RADIATION DAMAGE TO CELLULAR MEMBRANE AS STUDIED BY MAGNETIC RESONANCE AND FLUORESCENCE TECHNIQUES

Srinivasan, V. T., Singh, B. B., Verma, N. C., Mishra, K. P., Bhabha Atomic Research Centre, Biology & Agriculture Div., Trombay 400085, Bombay, India

The implication of cellular membrane in radiation damage suggests the possibility of this membrane damage being used as

a measure of absorbed dose in radiation accidents. As a sample of blood is perhaps the most commonest one easily available, the membrane fragility of red blood cells has been used as a criterion of radiation damage.

From the dosimetric point of view, the radiation induced hemolysis of red blood cells under isotonic conditions does not seem promising as hemolysis is induced only at high doses (16 to 50 krad). However post-irradiation incubation under hypotonic condition results in significant hemolysis even at lower doses. By carrying out post-irradiation incubation at higher temperature (37 degrees C) doses as small as 1 krad can be measured and there is good linearity between the percentage hemolysis and radiation dose.

In order to measure smaller doses, it is proposed to amplify the radiation effect on membranes, if possible, by post-irradiation treatment of cells with membrane specific drugs.

273. ABORTIVE TESTS WITH FLUORESCENT PROBE MOLECULES FOR X-RAY-INFLICTED DAMAGE IN THE SURFACE MEMBRANES OF CLL LYMPHOCYTES (ABBREV)

Peel, W. E., Vaughan Smith, S., Thomson, A. E., Imperial Cancer Research Fund, Cytochemistry Laboratory, Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom

According to Yonei and Kato (Radiation Research, 1978, 75, 31) 'X irradiation readily induces significant changes of erythrocyte membrane structure.' They based this conclusion on the behavior of the fluorescent probe molecules 1-anilinonaphthalene-8-sulphonate (ANS) and diphenylhexatriene (DPH) incorporated into the membrane of red cell ghosts. The changes in behavior observed were interpreted as responses by the probes to reductions in intramembranal microviscosity resulting from X-ray-induced changes in conformation of membrane proteins. A dose as low as 100R was reported to be effective. This dose is in the range of sensitivity to the lethal action of X-rays shown by human non-dividing lymphocytes, in which cells the plasma membrane has been postulated as the primary target (Report 228).

Our first set of experiments were conducted on CLL-lymphocytes. Using DPH we could find no evidence of change in degree of fluorescence depolarization ('P') - the measure of microviscosity used by Yonei and Kato - for cells incubated 1 hr. or 4 hrs. after supra-lethal doses of 500 R or 4000 R. Elevations in P value (increases in microviscosity) and in fluorescence intensity were recorded with ANS and another probe, 2-p-toluidinonaphthalene-6-sulphonate (TNS) for cells incubated 4 hrs. after a dose of 4000 R. But these changes could be explained entirely by the presence of dead (pyknotic) lymphocytes, which can additionally incorporate ANS and TNS intracellularly.

The second set of experiments was a repeat of those of Yonei and Kato on red cell ghosts. We failed to reproduce their observations. In fact, slight increases in P value were recorded after irradiation with 4000 R using ANS and TNS (but not DPH).

The changes in P value reported by these authors as a result of X irradiation are as great (about 5 percent) as the errors of the method of measurement in our hands.

274. THE PLASMA MEMBRANE AS A POSSIBLE TARGET FOR THE LYMPHOCYTOCIDAL ACTION OF X-RAYS

Vaughan Smith, S., Peel, W. E., Thomson, A. E., Imperial Cancer Research Fund, Cytochemistry Laboratory, Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom

It remains unexplained why non-dividing lymphocytes are much more sensitive to being killed by ionizing irradiation than other types of somatic cells. That DNA may not be the primary target is indirectly suggested by the lack of difference in lymphocyte radiosensitivity to X-rays or neutrons. This is in contrast to dividing mammalian cells where there are such differences expressed that correlate with the extent of damage to DNA.

The plasma membrane may be the primary target. Increased membrane permeability is the earliest recorded effect on thymocytes of lethal doses of irradiation. The radiation-induced killing of non-dividing lymphocytes is more readily potentiated by oxygen than is that of other mammalian cell types. The oxygen effect in bacteria can be attributed to peroxidation of

cell membrane lipids. Decreased radiosensitivity may be conferred upon mutants of *Escherichia Coli* unable to synthesize or metabolize unsaturated fatty acids by feeding them polyunsaturated fatty acids (oleic or linolenic). These are incorporated into the cell membrane.

It has been found here previously that healthy persons' lymphocytes contain more of the polyunsaturated fatty acyl chains than chronic lymphocytic leukemia lymphocytes (ICRF Scientific Report 1977 - report 209). The relatively greater radiosensitivity of the leukemic cells may be due to reduced availability of unsaturated fatty acyl chains to inactivate damaging free radicals produced in the phospholipid membrane bilayer during irradiation.

Several types of experiments are planned. (1) Incorporation of lipid-soluble anti-oxidants into the plasma membrane to block peroxidation chain reactions. (2) Use of specific blocking reagents for cell surface thiol groups to prevent their interaction with damaging free radicals produced by ionizing irradiation. (3) Modulation of lymphocyte surface membrane fluidity (e.g., by insertion or extraction of cholesterol). It is known that changes in radiosensitivity of bacteria can be partially correlated with variations in membrane fluidity.

275. RADIATION INJURY IN SUBPOPULATIONS OF LYMPHOCYTES

Anderson, R. E., Scaletti, J. V., Standerfer, J. C., University of New Mexico, School of Medicine, Dept. of Pathology, University Hill N.E., Albuquerque, New Mexico, 87131, United States of America

Subpopulations of lymphocytes may be defined via functional, morphological and immunochemical differences. Included among the properties that distinguish thymus-derived (T) vs. bone marrow-derived (B) lymphocytes are the apparent presence or absence of receptor sites for specific substances and the response of the involved cell type to interaction with mitogens. These differences between T and B cells reflect discrepancies in the chemical composition and structural arrangements of the plasma membrane.

B cells may be further divided into subpopulations on the basis of surface immunoglobulin. More recently, subpopulations of T cells have been defined on the basis of function and cell surface alloantigens; helper (Ly 1 positive 2 negative 3 negative) and suppressor (Ly 1 negative 2 positive 3 positive) cells are of particular importance.

Investigations, to date suggest that most, if not all, the above subpopulations differ in their sensitivity to ionizing radiation. Thus: 1) irradiated B cells placed in tissue culture survive less well than T cells; 2) the capacity of irradiated cells to traffic in normal fashion is inhibited at lower dose levels for B cells than T cells; 3) after whole body exposure, proportionately greater numbers of T than B cells continue to recirculate and maintain viability; 4) B cells demonstrate radiation-induced morphologic alterations at lower dose levels than do T cells as documented by transmission and scanning electron microscopy; 5) T and B cells activated to appropriate mitogens or antigens in vivo and in vitro are more resistant than their nonactivated counterparts; 6) suppressor T cells appear to be more radiosensitive than helper T cells; 7) a subpopulation of cytotoxic T cells is very resistant to radiation injury.

The purpose of this project is twofold: expand the above observations on the radiosensitivity of defined populations of lymphocytes with particular attention to low dose effects; define the physical-chemical basis of these differences in radiosensitivity and thereby gain a better understanding of the pathogenesis of radiation-induced interphase death of lymphocytes.

6. EFFECT OF RADIATION ON CELLULAR GROWTH KINETICS

276. RADIATION INDUCED DEFECTS IN CELL KINETICS

Germer, E. W., University of Arizona, School of Medicine, Dept. of Radiology, 1501 N. Campbell Ave., Tucson, Arizona, 85721, United States of America

The use of radiotherapy in the treatment of cancer is based on the fact that ionizing radiation inhibits the proliferative capacity of tissues and results in cell death. The objective of this proposal is to study the effects of radiation in a related sequence of biochemical events which occur during the mammalian cell

cycle and are involved in cell proliferation control. Previous studies have shown that the non-histone chromosomal proteins may be very important in the control of DNA replication, gene activation and cell division. The application proposes to study the effects of radiation on these proteins, as well as the enzymes and factors, specifically the protein kinases and cyclic nucleotides, which modify these proteins and possibly affect their regulatory functions. These studies should add fundamental insight to the understanding of radiation induced defects in cell cycle kinetics, and ultimately contribute to the optimization of the use of radiation for the treatment of human neoplastic disease.

277. X-RAY DOSE-RATE EFFECTS ON CELL KINETICS

Schwarz, G., St. Vincent's Hospital & Medical Center of New York, 153 W. 11th St., New York, New York, 10011, United States of America

The objective of this study is to obtain a detailed picture of cellular kinetics and lethality under conditions of continuous irradiation and to investigate whether and how the effects of continuous irradiation can be predictably reproduced by fractionated treatment in four different in vitro cell lines.

The dose-rate effect due primarily to the accumulation of radiation damage and repair was studied by continuous irradiation at various dose rates at temperatures which slow down or prevent the movement of cells through the cycle. These studies will also include stationary cell populations in plateau phase.

Dose-rate effects due to differences in the characteristics of the mitotic cycle will be studied by incubating cells during irradiation at temperatures between 28 degrees C and 37 degrees C, a procedure which essentially does not affect the ability of cells to accumulate and repair radiation damage, but which multiplies the duration of the mitotic cycle several times.

The potential advantages of combining mild (39 degrees C to 42.5 degrees C) hyperthermia with continuous irradiation, as well as the effects of short (45 degrees C to 46 degrees C) heat treatments followed by continuous irradiation at various temperatures will be studied.

278. CELL KINETICS OF IRRADIATED TUMORS

Goldfeder, A., New York University, Washington Square & University College of Arts & Sciences, Dept. of Biology, 421 1st Ave., New York, New York, 10012, United States of America

The aim of this renewal project is to apply detailed information on morphologically defined G1 phase cells and their cycling counterparts, acquired during studies reported in this application. Planned studies will be conducted in vivo on transplantable lines of murine mammary carcinomas and sarcomas, and on lines of human mammary carcinomas propagated in athymic-nude mice.

Studies will be concerned with several problems: 1) The capacity of G1 phase-confined cells to recover from sublethal or potentially lethal radiation damage; 2) comparison of both types of repair in vivo; 3) exploration of a possibility that repair of radiation damage in some instances might be an artifact caused by dispersion procedures of tested tumor cells; 4) relationship between hypoxic cells and G1 phase-confined or proliferating cells; 5) kinetics and clonogenic potential of the 'fast' and 'slow' components of G1 phase-confined cells in untreated as well as in irradiated tumors; 6) automatization of nucleolar screening in tumor cells; and 7) application of diffusion chamber-semisolid medium technique to human carcinomas. Since these experiments will be performed on mouse tumor systems as well as on human mammary carcinomas, the clinical implication is inherent.

The overall goals of the proposed research are: a) To extend the current knowledge of G1 phase-confined cells in experimental as well as in human tumors under in vivo conditions, b) to develop an in vivo experimental testing and screening system applicable to human cancers, and c) to apply this system to further exploration of combination therapy.

279. CONTROLS OF CELL CYCLE AND CELL PROLIFERATION

Naha, P. M., Paterson Lab., Dept. of Cell Biology, 550 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom

OBJECTIVE: There are indications that differentiation and carcinogenesis are related to the regulatory changes in a cell which are possibly determined during G1 phase of the cell

cycle. It has been suggested that there are some rate controlling signals in G1 itself and at the G1/S boundary which have regulatory function. Our objective is to decipher the controlling functions in G1.

APPROACH: Temperature-sensitive (ts) variant clones of BALB/c-3T3 were put on a linear time scale by measuring their time of entry into S phase (Nature, 258, 49-53, 1975). Complementation tests between the ts variants suggested (J. Cell Science, 35, 53-58, 1979) that there are at least three complementation groups, any of which could block a cell's entry into S phase. Initial biochemical studies suggested the presence of an additional non-histone protein in the ts variants blocked either early or late in the G1 phase (mol. wt. 24,000). This raised the possibility of a negative regulatory control of cell proliferation.

PROGRESS: Experimental conditions have been developed (J. Cell Science, in press) to induce G1 negative cells at high frequency by UV-irradiation of ts cells arrested in G1 phase at the restricted temperature. Neither the wild type nor the ts variants could be 'mutated' under similar conditions. Such G1 negative cells might be useful in the search for relevant 'repressor' protein controlling cell cycle proliferation.

280. BIOCHEMISTRY AND RADIATION EFFECT DURING THE CELL CYCLE

Helmstetter, C. E., Roswell Park Memorial Inst., Dept. of Experimental Biology, 666 Elm St., Buffalo, New York, 14263, United States of America

The duplication process of *Escherichia coli* will be investigated, with emphasis on the mechanisms which control the frequency of chromosome replication and cell division. The following projects will be undertaken to investigate the molecular mechanisms involved in initiation of chromosome replication: 1) Analysis of the effects of oriC-containing plasmids on host cell growth, 2) Determination of the timing of oriC plasmid replication during the division cycle of *E. coli* B/r, 3) Studies on the requirements for RNA and protein synthesis in oriC plasmid replication, 4) Examination of cloned chromosomal fragments which suppress temperature-sensitive dnaA mutants, 5) Analysis of the interaction between the dnaA gene product and RNA polymerase during initiation of chromosome replication, and 6) The kinetics of synthesis of DNA-binding proteins with respect to initiation and elongation of the *E. coli* chromosome.

BIBLIOGRAPHIC REFERENCES: Tang, M.S., and Helmstetter, C.E. Coordination between Chromosome Replication and Cell Division in *E. coli* B/r. *J. Bacteriol.*, 1980, in press. Leonard, A.C., Weinberger, M., Munson, B., and Helmstetter, C.E. The Effects of oriC Plasmid Maintenance on Host Cell Growth. ICN-UCLA Symposia on Molecular and Cellular Biology Vol. XIX. Eds., B. Alberts and C.F. Fox, Academic Press, New York, 1980, in press.

281. CELL PROLIFERATION IN EPITHELIAL TISSUES - GROWTH CONTROL, CARCINOGENESIS AND RESPONSE TO IRRADIATION AND CYTOTOXIC CHEMOTHERAPY

Wright, N. A., Cancer Research Campaign, Cell Proliferation Unit, Fulham Palace Rd., c/o Charing Cross Hospital, London W6 8RF, England, United Kingdom

OBJECTIVES: a) To identify and quantify stem cells in the small intestinal crypt. b) To analyze aspects of the control of cell proliferation in the intestine with special reference to the size of the functional compartment. c) An analysis of cell line specific growth control substances in the intestine. d) The evolution of clonogenic tumor cells during carcinogenesis in the intestine. e) The selectivity of cell death in proliferating and resting in vivo cell populations.

APPROACH: The approach will be mainly via orthodox cell kinetic techniques involving metaphase arrest, microdissection and autoradiography, and clonogenic assays involving microcolony analysis of the intestine and in the prostate gland.

282. DNA REPAIR AND RECOVERY IN THE MAMMALIAN CELL CYCLE

Humphrey, R. M., Clarkson, J. M., Adair, G. M., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., Dept. of Physics, 6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas, 77030, United States of America

The ultimate objective of this research is to determine the biochemical basis of cell cycle-dependent variations in the cytotoxicity and mutagenicity of radiation and chemical agents, and to define the relationship of certain DNA repair processes to mammalian cell survival and mutagenesis. We propose to determine: a) the extent to which cell cycle-dependent variations in cell killing by mutagenic agents are paralleled by variations in induced mutation frequencies, b) whether cell killing and mutation induction by such agents display qualitatively similar dose-response kinetics, and c) the effects of specific DNA repair processes, dose-fractionation and inter-dose recovery time on the kinetics of repair and ultimate expression of potentially-lethal versus potentially-mutagenic lesions. A second major objective of this research is to isolate and characterize a broad spectrum of different mutagen-sensitive variants of CHO cells that could be used to investigate the molecular and genetic bases of DNA repair and somatic cell mutation.

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283. EARLY AND LATE EFFECTS OF RADIATION OF DIFFERENT QUALITY AND AT DIFFERENT DOSE RATES

Baum, J. W., Rohrig, N., U.S. Dept. of Energy, Brookhaven National Lab., Upton, New York, 11973, United States of America

The primary objective of this program is the accumulation of experimental information for the development and evaluation of theoretical models of the mechanisms involved in the induction of biomedical effects by ionizing radiation. Effects under study in eukaryotic cells, tissues, and whole organisms include cell killing, mutation, chromosomal aberration production, transformation, tumor induction, and DNA strand breakage. Quantitative and qualitative variations in biological response as a function of the microscopic distribution of energy deposition provide significant insights into the action of radiation, and often of other physical and chemical agents as well. Irradiation of monolayers of mammalian cells with monoenergetic charged particles provides a minimum spread of radiation quality over a large and interesting range of lineal energy deposition. Response to radiations of such defined quality, dose, and dose rate at different stages in the cell cycle must be accounted for by any theory of the mechanisms of radiation biology. For exposure of large organisms and for more applied purposes (regulatory and cancer considerations) monoenergetic neutrons provide a convenient high LET radiation modality with defined spectra of radiation quality. A dedicated Van de Graaff generator makes possible the production of these radiations over a wide spectrum of energies.

284. HEAVY ION RADIATION AND MULTICELLULAR SPHEROIDS

Alpen, E. L., Rodriguez, A., University of California, Berkeley Campus, Lawrence Berkeley Lab., Donner Laboratory, Berkeley, California, 94720, United States of America

We propose to examine the relative biological effectiveness of heavy ion radiation on cell lines grown as multicellular spheroids. These spheroids have some properties associated with organized tissue systems. We will examine the mechanisms underlying the apparent fraction of radioresistant cells in spheroids. The role of cell cycle redistribution and anoxia will be evaluated and potential for repair of sub-lethal damage and potentially lethal damage will be determined. The equivalent of a 'tumor cure dose' for this in vitro system will be measured, along with growth delay as a function of LET of the heavy ion radiation. Alteration of cell cycle distribution in regrowing tumors will also be evaluated.

7. OTHER EFFECTS OF RADIATION ON SPECIFIC CELLS AND TISSUES

285. EARLY AND LATE RADIATION DAMAGE IN MOUSE LUNG

Travis, E. L., Fowler, J. F., DeLuca, A. M., Sanders, C. J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Radiobiology Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

Radiation causes two distinct histological changes in the mouse lung which occur at clearly separated times; radiation pneumonitis at 4-5 months after treatment (early damage) and fibrosis at longer than 9 months post treatment (late damage). It is possible to dissociate these 2 types of damage, i.e., cause little early reaction while inducing a severe late reaction. This suggests that 2 different cell types might be responsible for the 2 types of damage. It is the purpose of this study to study the conditions under which early and late damage can be dissociated, to define the radiobiological characteristics of the 2 types of damage, and define those cells which are involved in each type of damage. Answers to these questions may enable us to reduce late damage in the lung.

286. RADIATION PRODUCED HUMAN PULMONARY LESIONS

Rubin, P., University of Rochester, School of Medicine & Dentistry, Dept. of Medicine/Radiology/Surgery, 601 Elmwood Ave., Rochester, New York, 14627, United States of America

This is part of a broader project. A summary of this subproject is not available.

287. VISCOELASTIC ANALYSIS OF BRAIN AND TUMOR DNA

Shafer, R. H., Wun, K. W., University of California, San Francisco Campus, School of Pharmacy, Dept. of Pharmaceutical Chemistry, 551 Parnassus Ave., San Francisco, California, 94122, United States of America

This research project is concerned with the viscoelastic behavior of mammalian cell DNA. We are examining the conformational properties of mammalian DNA from cells that have been treated with gamma rays and various drugs in an effort to characterize the DNA damage resulting from such treatment. Repair of the various lesions introduced by gamma rays and drugs is also being investigated. The types of damage under study include single-strand and double-strand breaks and DNA-DNA crosslinks.

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288. DELAYED EFFECTS OF RADIATION

Casarett, G. W., University of Rochester, School of Medicine & Dentistry, Dept. of Radiation Biology & Biophysics, 601 Elmwood Ave., Rochester, New York, 14627, United States of America

This project is a systematic, multidisciplinary investigation, in mammals in-vivo, of: (1) the nature and mechanisms of development of the early unreversed or residual radiation injuries in cells, tissues, and organs; (2) the direct and indirect mechanisms by which these injuries lead in time to permanent and delayed radiation damage; (3) the manner in which this damage is involved in the pathogenesis of radiation-induced or temporally-advanced degenerative and neoplastic diseases, premature aging, and life-span shortening; (4) the nature and extent of the influence of damage or response of organs and tissues on the damage and recovery processes in other organs and tissues and the body as a whole following irradiation in various modalities (with respect to body parts irradiated, dose, and dose rate); and (5) postirradiation treatments for prevention or reduction of the permanent and delayed radiation effects. The correlated, multidisciplinary methodology (subcellular, cellular, histologic, histochemical, biochemical, physiological, angiographic, radiographic, pathological, immunological, actuarial) is applied periodically in coordinated fashion to sacrifice groups and longitudinal study groups of animals to elucidate mechanisms of radiation effects and of natural disease development and aging from sequential events and processes occurring in parenchymal cells, supporting

connective tissue cells and matrices, and vasculature and circulation throughout the life span.

B. MODIFICATION OF THE BIOLOGICAL EFFECTS OF RADIATION

1. RADIOPROTECTORS AND RADIOSENSITIZERS

289. CAROTENOIDS AS ANTI-TUMOR AGENTS FOR SKIN TUMORS

Mathews Roth, M. M., Murphy, J. C., Krinsky, N. I., Harvard University, Boston Campus, Peter Bent Brigham Hospital, 721 Huntington Ave., Boston, Massachusetts, 02115, United States of America

Light, particularly the sunburn spectrum (UVB, 280-320 nm), has been implicated as an etiologic agent in the induction of skin tumors in animals and man. We have shown that the administration of carotenoid pigments to patients with erythropoietic protoporphyria can ameliorate the light sensitivity associated with this disease, and that the administration of these pigments to normal volunteers was found to have slight but statistically significant action in delaying the appearance of erythema caused by the sunburn range. We have also found that phytoene, a precursor pigment having absorption maxima in the sunburn range, significantly decreased the erythral reaction in guinea pigs exposed to this radiation. In addition, we and others have shown that carotenoid pigments can inhibit the formation of free radicals which have been implicated in photocarcinogenesis. Therefore we have studied the effect of the systemic administration of several carotenoid pigments on the development of skin tumors induced by sunburn radiation and topical carcinogens in hairless mice.

We have found that the administration of beta-carotene, phytoene or canthaxanthin causes a delay in development, and inhibits significantly the development of tumors induced by UV-B (290-320 nm) per mouse, in hairless mice receiving these pigments. In addition, beta-carotene has an inhibitory effect on dimethyl benz (a)anthracene-induced tumors, but phytoene and canthaxanthin have no such effect. We now propose to extend our studies in two directions; 1) to investigate the possible mechanisms of action of carotenoid protection, by determining photo-product formation on DNA and cellular damage in animals receiving each of the pigments as compared to animals receiving placebo, and to study some aspects of the pharmacokinetics of these pigments, either alone or in combination, to establish the most effective administration methods for maximal tumor inhibitory effect. In addition, we plan to study the effect of crystalline preparations of the water soluble carotenoid, crocetin and other carotenoids as they become available, on the effect of UV as well as chemically-induced skin tumors.

290. POTENTIAL BIOGENIC MECHANISM(S) AND SYSTEMIC PROPHYLAXIS FOR ACTINIC SKIN CANCER

Black, H. S., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, 2002 Holcombe Blvd., Houston, Texas, 77031, United States of America

That diet plays an important role in predisposition to cancer is becoming increasingly apparent. We have previously demonstrated that a number of antioxidants and enzyme inducers, when added to the diet of experimental animals, modify the carcinogenic response to UV-radiation and markedly reduce the incidence of squamous cell carcinomas. Among those compounds, butylated hydroxytoluene, a phenolic antioxidant widely used in this country as a food preservative, was quite effective. In addition we have shown that dietary cholesterol has an inhibitory effect upon development of UV induced tumors. Although other lipids are known to modify the incidence rate of certain types of chemically induced and spontaneous tumors, we have demonstrated such a role for dietary fat in UV-carcinogenesis. Data from nutritional studies employing hairless mice indicate that state of lipid saturation modifies the carcinogenic response to UV and suggest that levels of dietary fat may alter antioxidant mediated modification of UV-carcinogenesis. It may be concluded that adherence to dietary standards is as important as other experimental parameters when comparisons of carcinogenic potentials are to be made. Further,

when the Ames mutagenicity test was employed to examine effect of diet upon 2-AAF (a potent hepatocarcinogen) activation, it was shown that dietary antioxidants markedly enhanced the mutagenic frequency. Dietary fat, upon challenge with 2-AAF, also enhanced activation of this carcinogen. Thus dietary fat plays an important role in predisposition to both physical and chemical carcinogenesis. In addition beneficial modification of one type of carcinogenic agent with antioxidant supplementation may predispose to another.

291. DETERMINANTS OF RESISTANCE TO IONIZING RADIATION AND ALKYLATING AGENTS IN NORMAL AND LEUKEMIC BLOOD CELLS

Bigley, R. H., Oregon State Higher Education System, University of Oregon Health Sciences Center, School of Medicine, Dept. of Medicine, 3181 S.W. Sam Jackson Park Rd., Portland, Oregon, 97201, United States of America

Our research has led us to the concept that ascorbate, maintained by ascorbate reducing enzyme(s), is an important determinant of radioresistance. This proposal is designed to examine the role of this activity, along with other potential inactivators of oxidants and free radicals, in normal and neoplastic WBC; and to explore the possibility that radiosensitivity of different kinds of cells can be differentially altered.

Biochemical considerations suggest that reduced ascorbate and reduced glutathione react readily with alkylating cations. We propose to explore the possibility that these molecules are determinants of resistance to cell injury by alkylating agents.

Initial studies will utilize normal and chronic leukemic lymphocytes and granulocytes and normal macrophages, as examples of cells with differing sensitivities to radiation and/or alkylating agents. Cell damage by these agents will be measured; this will be correlated with cellular ascorbate reducing capacity and content of selected antioxidants and free radical inactivators before and after exposure to X-radiation or alkylating agents.

Using selected animals and animal cells, we will try to alter WBC radiosensitivity differentially by induction or repression of enzyme synthesis and by changing cell content of antioxidant molecules.

Identification of major determinants of resistance to radiation or alkylators should lead, as our studies already promise, to medically practicable means for selective alteration of cell tolerance to radiation or alkylators. This in turn should lead to more precise and effective treatment of leukemia and lymphoma, and immunosuppression.

292. BIOCHEMICAL MECHANISMS OF RESISTANCE TO IONIZING RADIATION OF NORMAL AND LEUKEMIC LYMPHOCYTES

Rigas, D. A., Oregon State Higher Education System, University of Oregon Health Sciences Center, School of Medicine, Dept. of Biochemistry, 3181 S.W. Sam Jackson Park Rd., Portland, Oregon, 97201, United States of America

Blood lymphocytes are among the most radiosensitive cells while blood granulocytes (PMN) are most radioresistant. Both suffer interphase death following exposure to ionizing radiation. The determinants of radioresistance and the nature of injuries leading to interphase death of these cells are not clearly defined. Our work, as well as observations made by others, led us to the view that membrane damage due to the autocatalytic process of lipid peroxidation is a principal cause of interphase death; that the ability of the cell to scavenge and inactivate free radicals, oxidants and lipid peroxides, and therefore its content of the involved enzymes, as well as its capacity to maintain antioxidants such as ascorbate and glutathione in the reduced form are probably critical determinants of the radioresistance of the cells. Objective measures of radioresistance and of parameters of radiation-induced cell injury will be made. The objective is to correlate the measured radioresistance of the cell with its capacity to scavenge free radicals and maintain antioxidants reduced, and to determine whether membrane damage due to lipid peroxidation is the principal cause of interphase death.

We propose to isolate and use normal and leukemic lymphocytes untransformed, and mitogen transformed and granulocytes of differing radiosensitivities to study the above hypothesis. The radioresistance (D37), lipid peroxidation and other damage of X-irradiated cells will be measured. These will be correlated with measurements before and after X-irradiation of:

(1) the cellular content of antioxidants and free radical scavengers (superoxide dismutase, catalase, glutathione peroxidase, ascorbate, reduced glutathione, alpha-tocopherol); (2) the capacity of the cell to maintain in the reduced form A and GSSG.

Elucidation of the damage inflicted by ionizing radiation to biomolecules and of the major determinants of radioresistance of blood cells should help to understand the mechanism of interphase death; to make more precise, rational therapeutic decisions in leukemia, lymphoma and immunosuppression, and potentially develop ways of altering the radiosensitivity of cells and improve the radioprotective, radiodiagnostic and radiotherapeutic methods.

293. INVESTIGATION OF FACTORS THAT CONTROL INTRINSIC AND ACQUIRED RESISTANCE TO CYTOTOXIC AGENTS

Fox, B. W., Carr, F. J., Bedford, P., Garner, A., Paterson Lab., Dept. of Experimental Chemotherapy, 530 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom

OBJECTIVE: To understand the underlying mechanisms of both intrinsic and acquired resistance in tumor cells towards radiation and antitumor agents, e.g., alkane sulphonates, antifolates, anthracyclines.

APPROACH: By studying temporal aspects of DNA repair, detailed mode of resistance to, e.g., busulphan, flow cytometry investigation of sub-cellular populations with regard to their changing biochemistry following treatment.

PROGRESS: Flow cytometric analysis of daunorubicin interaction with the cell and DNA, fluorescent methotrexate interaction. Measurement of temporal aspects of DNA repair activity and its relationship to cell cycle perturbations. The influence of medium conditioning.

294. COMBINED EFFECTS OF RADIATION AND CYTOTOXIC DRUGS ON THE RADIATION TOLERANCE OF THE CENTRAL NERVOUS SYSTEM

Hopewell, J. W., Karbal, A., University of Oxford, Research Inst., c/o Churchill Hospital, Oxford, England, United Kingdom

OBJECTIVE: Chemotherapeutic agents, in particular methotrexate, are currently being used in conjunction with brain irradiation as part of the prophylactic treatment of children with leukemia. It is proposed to examine the possible effects of various drug combinations on the radiation tolerance of the central nervous system.

APPROACH: It is proposed to investigate the mechanisms responsible for the development of damage in the brain and spinal cord in experimental animals. Effects will be evaluated by the quantitative assessment of cellular changes in histological material obtained by the serial killing of treated animals. The importance of field size will be investigated.

Modifications in normal tissue and tolerance produced by radiation of the spinal cord in combination with various dose levels of intrathecal methotrexate and other drugs will be determined by establishing the proportion of animals developing paralysis after 6 months. Iso-effect doses will be compared to determine the relative changes in cord tolerance.

In the brain, radiation depletion of the cells in the subependymal plate region will be used as a measure of radiation effects. Modifications in this response, produced by drug combinations, will be evaluated.

295. RADIATION PROTECTION ASPECTS IN MAMMALIAN PRENATAL DEVELOPMENT

Michel, C., Balla, I., Fritz Niggli, H., Universitat Zurich, Medizinische Fakultät, Ramistrasse 100, 8006 Zurich, Switzerland

OBJECTIVE: Evaluation of low-dose radiation effects on embryonic development. Study of the influence of dose-modifying factors or conditions. Comparison of the biological effectiveness of different kinds of ionizing radiation.

APPROACH: Experimental investigations using mouse embryos at various stages of development. Combined treatment with chemicals and radiation (X-rays, high energy photons and electrons, pions) and evaluation at different times following exposure. Macroscopic and microscopic analysis of embryonic damages, such as resorption, death, malformation, growth retardation and late effects (cataract formation, tumor induction). Study of pathogenetic mechanisms (chromosomal aberrations and cell death) and search for correlations between cellular changes and macroscopically visible defects.

PROGRESS: Significant effects on embryonic development could be demonstrated following irradiation with only 0.01 Gy (1 rad). Decreasing effectiveness of radiation in the order: pions more effective than X-rays more effective than electrons was found as well as radiosensitizing effects with iodoacetamide, tetracyclines and lucanthone or misonidazole. The experiments on life-shortening and tumor induction are still in progress. The microscopical analysis showed radiation-induced decrease in the proliferation rate and changes in the mitotic phase ratio.

296. RELATIONSHIP OF INTRACELLULAR CYCLIC NUCLEOTIDE CONCENTRATION TO RADIOSENSITIVITY

Lehnert, S. M., McGill University, Royal Victoria Hospital, Dept. of Therapeutic Radiology, 687 Pine Ave. W., Montreal, Quebec, H3A 1A1, Canada

OBJECTIVE: To determine relationship of intracellular cyclic nucleotide levels to post-irradiation survival.

APPROACH: In vivo experiments the effects of cyclic AMP phosphodiesterase inhibitors on radiation response of intestine, bone marrow, skin, EMT6 adenocarcinoma and KHT fibrosarcoma are being studied. The effect of inhibitors of cyclic nucleotide phosphodiesterase, adenylyl cyclase agonists and cyclic AMP analogs on post-irradiation survival, DNA synthesis and cytoskeletal organization are being investigated in cultured CHO cells.

PROGRESS: Diethyl amino reserpine (DL-152), an inhibitor of cyclic AMP phosphodiesterase, has been found to protect skin and intestine but not bone marrow against X-rays. Clonogenic cells of the KHT tumor are reduced in number by exposure to DL-152 but the cells surviving this treatment are more radioresistant than untreated cells. In vitro, compounds interacting with cyclic AMP metabolites have been found to have a dual effect on radiation response, involving two separate mechanisms. One, dependent on the presence of elevated cyclic AMP post-irradiation leads to increase in the slope of the survival curve, while the other, independent of cyclic AMP concentration per se, causes enhancement of the extent of the shoulder on the survival curve.

297. RADIATION SENSITIZATION APPLIED TO CANCER RADIOBIOLOGY

Epp, E. R., Michaels, H. B., Massachusetts General Hospital, Division of Radiation Biophysics, 32 Fruit St., Boston, Massachusetts, 02114, United States of America

The mechanisms by which oxygen and chemical radiosensitizing agents act in irradiated mammalian cells will be studied, both independently and interactively. Aspects of the nature of radiation sensitization will be examined in detail in CHO cells under conditions where both hypoxic cell sensitizers and low levels of oxygen are present. In the clinical application of sensitizers such as the nitroimidazoles, e.g., misonidazole, to cancer radiotherapy, it is likely that there will be an important subpopulation of tumor cells that contain a low concentration of oxygen, but are not radiobiologically hypoxic. The effects of sensitizers on such cells will be determined in this project. The nature of the interaction of oxygen and chemical sensitizers has yet to be identified: the agents may act independently or in a complex additive manner. Also to be investigated is the possibility of an additive or synergistic effect between radiosensitization and the specific hypoxic cell toxicity recently observed with many sensitizer compounds. This study will evaluate these two factors which are of importance relative to the optimum application of sensitizers in fractionated radiotherapy. Experiments to be performed using high dose rate techniques will provide information on the kinetics and mechanism of sensitization and of the related process of radiolytic oxygen depletion, known to occur in mammalian cells containing low levels of oxygen, which may play an important role in radiotherapy for solid tumors. It is expected that these data will be useful in identifying site(s) of action of oxygen and other sensitizers and therefore aid in the formulation of the molecular mechanism for radiosensitization. Also to be studied, using the high dose rate radiation capability, are several other modifiers of radiation damage, namely 'shoulder-modifying' sensitizing agents, and compounds believed to alter the indirect action damage to DNA. These studies will provide information on the mechanisms of radiation damage,

especially pertaining to the effects of oxygen and radiosensitizers, which are important current concerns in radiobiology and cancer radiotherapy.

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298. HYPERTHERMIC OXYGENATION AS RADIATION SENSITIZER

Bicher, H. I., Hetzel, F. W., Henry Ford Hospital, Dept. of Therapeutic Radiology, 2799 W. Grand Blvd., Detroit, Michigan, 48202, United States of America

Preliminary physiological experiments demonstrated a rise in tumor TpO₂ using short term (10 min) localized mild hyperthermia (39-41 degree C) both in animals and humans. The mechanism of this effect conceivably could be mediated through an increase in blood flow to the cancerous tissue. Associated effects on metabolism and intercellular pH remain to be determined. We propose to measure concomitantly TpO₂, pH, local blood flow and tissue temperature using oxygen and pH ultramicroelectrodes (tip 1-5 micron), the local H ion generation method and microthermocouples respectively. Other functions such as blood pressure and body temperature will also be monitored. The objective is to establish the physiological mechanisms of observed rise in TpO₂, as well as to document it in different tumors at several host organ sites in various species. The ratio between the increase in O₂ tension in tumor and that in normal host tissue will also be studied.

Simultaneous radiobiological experiments will be performed to establish if this hyperthermic tumor oxygenation can be used as an adjuvant to radiation therapy. The tumor system in the initial studies will be a transplantable mouse mammary adenocarcinoma implanted into the upper hind limb of C3H mice. A holder has been designed which will hold 4 animals simultaneously with only the tumored portion of the legs exposed. For any given radiation dose the 4 animals will be treated as follows: 1) air, no heat; 2) air, heat; 3) oxygen, no heat; 4) oxygen, heat. Each dose point will consist of a total of 10-12 animals per experimental group (i.e. 40-48 animals per dose) for each of 10 doses. The simultaneous inclusion of the 3 control groups should serve as necessary and sufficient controls. The animals will then be followed 3 x per week for both tumor and normal tissue response throughout a period of 120 days/animal (assuming cure).

Based on the data obtained subsequent experiments will be performed employing an in vivo/in vitro WAG rat rhabdomyosarcoma to determine the cellular basis for the survival data obtained.

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299. SKIN-SENSITIZING AND CARCINOGENIC FUROCOUMARINS

Song, P., Tapley, J. K., Walker, E. B., Yoon, M. J., Texas Tech University, School of Arts & Sciences, Dept. of Chemistry, P.O. Box 4340, Lubbock, Texas, 79409, United States of America

Furocoumarins are skin photosensitizers and they are also carcinogenic in certain animal skins. The goal of the proposed study has been two-fold: (1) Elucidation of the molecular mechanism of photosensitizing potency of furocoumarins (psoralens), and (2) application of psoralens as molecular structural probes for nucleic acids and proteins. A variety of furocoumarin compounds will be used for these aims. These will be linked photochemically to lambda DNA and tRNA, as well as to proteins.

Attempts will be made to understand the ground state, dark binding, the mechanism of photo-induced modification of the nucleic acids and the consequences of it for biological activity. Thus, for example, we plan to examine the photobinding of psoralen to lambda DNA double helix to look for possible specificities of binding, to examine monoaddition versus cross-linking to the double helix and to see what effect such modifications have on the transcription of the DNA.

300. RADIATION AND CHEMICAL INTERACTIONS PRODUCING CELLULAR AND SUBCELLULAR DAMAGES AND THEIR REPAIR

Kada, T., National Inst. of Genetics, Dept. of Induced Mutation, Shizuoka-ken, Yata 1111, Mishima 411, Japan

In order to elucidate mechanisms of chemical sensitization of radiation-induced cellular lethal effects on normal and malignant cells, approaches are being made from the following point of view: 1) Radio-sensitization with iodine compounds such as iodoacetamide or 5-iodouridine might preferentially kill irradiated cells under mutagenesis. This may be useful to eradicate potential cancer cells. 2) Radiosensitization with hypoxic sensitizers of human cells and their repair mechanisms are studied using normal and Ataxia cells. 3) A number of typical hypoxic radiosensitizers such as Misonidazol are found to be DNA-damaging and mutagenic agents. Their strong toxicities may be due to this chemical background. This problem should be solved by screening other non-mutagenic active chemicals.

301. PHOTOCHEMISTRY OF DIMETHOXYCOUMARIN

Shim, S. C., Chae, K. H., Chi, D. Y., Korean Advanced Inst. of Science, Dept. of Chemistry, Cheong Yangni, P.O. Box 150, Seoul, Republic of Korea

Photoaddition of Dimethoxycoumarin to Thymidine: A mixture of DMC and thymidine (mole ratio 1:50) were irradiated with 350 nm light in frozen aqueous solution and in solid film state. The absorbance at 324 nm decreased more rapidly than in the case of irradiation of DMC alone. TLC analysis revealed 2 cross-addition products, one being a major product. A thin solid film gave the best results giving the largest quantity of the major product and benzene-acetone (2:3 v/v) solvent was the best eluent. The isolation and structure determination of photoaddition products of DMC to thymidine are in progress. The major photoadduct is very sensitive to 254 nm UV light giving DMC and thymidine and the adduct may very likely be a C4-cycloaddition product. The isolation of the product in large enough quantities for spectroscopic (UV, IR, NMR, mass) studies and elemental analysis is in progress by TLC and column chromatography. The structure of the cycloadducts will be a good model for furocoumarin-DNA photoaddition product and will be a great help in understanding the mechanism of photosensitization activity of furocoumarins.

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302. MOLECULAR ASPECTS OF ENERGY UTILIZATION, CELLULAR FUNCTION AND CARCINOGENESIS

Land, E. J., Swallow, A. J., Paterson Lab., Division of Biophysical Chemistry, 550 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom

OBJECTIVE: Improvements in our understanding of the mechanisms of carcinogenesis, whether induced by radiation or chemicals, are being pursued by physicochemical studies at the molecular level.

APPROACH: The incidence of radiations on cells results in the formation of free radicals and excited states. These ultimately cause changes in metabolism which in healthy cells can lead to transformation or cytotoxicity, and in malignant cells to cell death. Pulse radiolysis and flash photolysis are being used to follow the free radicals and excited states in cells specialized towards light absorption. Such information aids the interpretation of radiation-induced intermediates in skin photosensitization which can ultimately lead to carcinogenesis.

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2. INFLUENCE OF HORMONES

303. INTERACTIONS OF DRUGS, RADIATION AND HORMONES

Weichselbaum, R. R., Little, J. B., Hellman, S., Harvard University, Boston Campus, School of Public Health, Dept. of Physiology, 55 Shattuck St., Boston, Massachusetts, 02115, United States of America

Estrogen and other hormones are known to interact with both normal and malignant cells through specific receptor mechanisms and to effect changes in cell growth, division, and metabolism. The specific changes in cell cycle parameters, as well as the interaction of these hormones with radiation and cytotoxic drugs, are unknown. In this proposal we outline methods of examining the effects of estrogen and other hormones on viability as a function of cell cycle position, as well as the interaction of hormones and radiation and hormones and cytotoxic drugs on an in vitro human breast carcinoma line. To determine the in vitro kinetic parameters we will employ examination of growth curves and % labeled mitosis curves. Clonogenic survival curves and exponentially growing and plateau phase cultures will be employed to ascertain the effect of hormone, x-ray, and hormone, drug, and x-ray interactions in this cell line.

304. INFLUENCE OF REPEATED LOW DOSE IRRADIATION ON MAMMARY GLAND CARCINOGENESIS IN ESTROGENIZED RATS

Shellabarger, C. J., U.S. Dept. of Energy, Brookhaven National Lab., Upton, New York, 11973, United States of America

OBJECTIVES: It has been demonstrated that a synergistic interaction occurs between x-irradiation and estrogen on mammary adenocarcinoma formation in female rats of the AxC strain and, so far as is known, only in the AxC strain. It is the objective of this contract to study the effect of fractionation and protraction of the x-irradiation on this synergistic interaction. Three x-ray doses are to be studied, 80R, 40R, and 20R; and each of these doses to be given in 1, 4, or 8 fractions. Following the last irradiation, diethylstilbestrol (DES) pellets are implanted.

MAJOR FINDINGS: All rats have been entered into the study, have been followed for a maximum of 18 months, and have been terminated. Preliminary analysis of the 80R group discloses that DES given at 183 days of age is less effective than DES given at 393 days of age, at least in terms of the average number of mammary adenocarcinomas per rat. It also appears that DES given at 183 days of age and 80R of x-irradiation given at 181 days act in a synergistic fashion; but when the two agents were given at 391 and 393 days of age, no synergism was apparent during the first 250 days after DES treatment. Preliminary data from the 20R x 4 group and the 10R x 8 group show no lessening of the carcinogenic effect due to fractionation. Final analysis of all of the data will be completed within the next 3 months.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE: Because it has been reported that women exposed to repeated fluoroscopic examinations, women survivors of atomic bombings and women treated with x-ray for acute postpartum mastitis, all have an increased risk of developing breast cancer, radiation exposure is now associated with breast cancer of the human female. However, there is little animal data on the dose-response relationship of radiation and the risk of developing mammary neoplasia at low doses of radiation, and even less data on the possible sparing effect when the radiation is fractionated and protracted. It is the intent of this research to investigate the dose-response relationship and the effect of fractionation in an animal model in which synergistic interaction between x-rays and estrogen on mammary carcinogenesis has been demonstrated. (Text Abridged.)

305. RADIATION-INDUCED LEUKEMOGENESIS

vanBekkum, D. W., Central Org. for Applied Scientific Research T N O, Radiobiological Inst. T N O, 151 Lange Kleiweg, P.O. Box 5815, 2280HV Rijswijk, Netherlands

OBJECTIVES: To define the exact role of the thymus and its hormonal factors in the development of lymphomas after fractionated whole body irradiation. To analyze the mechanism of inhibition of lymphoma development in inbred irradiated mice with post-irradiation transplants of normal bone marrow.

APPROACH: Attempts to mimic the effect of thymic implants in thymectomized irradiated animals (restoration of lymphoma incidence) by thymus in diffusion chambers and by treatment with humoral thymic factors. Transplantation of bone marrow subpopulations (e.g., stem cells) into intact irradiated mice and study of lymphoma incidence.

PROGRESS: Isogenic bone marrow administered following 4 x 180 rad fractionated irradiation was found to effectively prevent the subsequent development of leukemia, confirming previous results of Kaplan et-al., Haran-Ghera et-al. and Ilbury et-al. However, in contrast to these authors who stated that only relatively large numbers of bone marrow cells are effective (5 times 10 to the 6th power - 3 times 10 to the 7th power/mouse), we observed a clear-cut protection by as little as 5 times 10 to the 5th power bone marrow cells/mouse. We also found that protection was exerted when the bone marrow was given 10 days after the last irradiation as opposed to 24 hours. Purified bone marrow stem cells (concentration factor 5 times) also protected irradiated mice against leukemia development, but so far we cannot say whether stem cell concentrates are more or less effective than unfractionated bone marrow.

Experiments with thymectomized animals, treated with newborn thymuses in diffusion chambers or with a long-term course of thymosin injections, are in progress and cannot yet be evaluated correctly.

306. INFLUENCE OF REPEATED LOW DOSE IRRADIATION WITH X-RAYS AND NEUTRONS ON MAMMARY GLAND CARCINOGENESIS IN THE RAT

Broerse, J. J., vanBekkum, D. W., Hollander, C. F., vanZwieten, M. J., Central Org. for Applied Scientific Research T N O, Radiobiological Inst. T N O, 151 Lange Kleiweg, P.O. Box 5815, 2280HV Rijswijk, Netherlands

The summary for this project is under entry no. 131.

307. ENDOCRINE CONTROL OF INDUCTION AND GROWTH OF EXPERIMENTAL MAMMARY TUMORS

Mouriquand, J. C., Jacrot, M., Laboratoire d'Histologie, 38700 La Tronche, France

OBJECTIVE: Endocrine control of DMBA induced mammary tumors in rats has been well described. The same approach is applied to mammary tumors induced in female Sprague-Dawley rats by fast neutron irradiations (14 MeV).

APPROACH: Single or fractionated low doses of fast neutrons are applied, at the onset of puberty, to virgin females under several pituitary gland stimulations. Endocrine control of the tumor bearing animal: estrogen and progesterone receptors of the tumors are compared with those obtained in DMBA induced tumors under similar pituitary stimulation. Two cell lines have been obtained from such tumors and are studied in vitro.

308. CELL SURVIVAL AND ONCOGENESIS AFTER IRRADIATION IN VITRO - THYROID EPITHELIUM

Gould, M., University of Wisconsin, Madison Campus, Medical School, Dept. of Human Oncology/Radiology, 450 N. Randall Ave., Madison, Wisconsin, 53706, United States of America

The goal of this project is to gain quantitative information on the number, nature and acute radiation response of the cells involved in initiation of the oncogenic process which leads to thyroid tumors following exposure of thyroid cells to ionizing radiation in vitro, and how this process is related to the type of radiation, the dose, and the hormonal exposure of the cells before and after irradiation. The project is divided into short-term studies devoted to development and application of the radiation dose-thyroid cell survival assay, and to long-term oncogenesis experiments. The former will involve preparation of monodispersed suspensions of thyroid epithelial cells, irradiation of the thyroid cells in vivo before preparation of suspensions or in vitro after suspension, and transplantation of known numbers

of cells into the subcutaneous fat pads of recipient rats. Initially, the recipients will be thyroidectomized and maintained on low-iodine diet and distilled water to elevate thyroid-stimulating hormone titers. The endpoint of the assay will be the determination of the number of untreated or treated thyroid cells necessary to establish a functionally or morphologically recognizable thyroid follicle unit. The effects of differing hormone balances in donor and recipient rats will be investigated later. Long-term studies will involve irradiation of thyroid cells in vivo or in suspensions in vitro which will then be similarly grafted, initially in thyroid hormone and iodine deficient animals. The animals will be observed for the development of thyroid neoplasm at the site of transplantation. By these methods, we hope to a) determine the total number of cells of the type from which the neoplasms are derived and which survive radiation exposure, b) minimize the abscopal effects of irradiation, and c) develop a system in which both physical and hormonal factors can be manipulated before and after exposure for the study of cell survival, repair and neoplasia in quantitative fashion.

309. RADIATION IN VITRO - MAMMARY NEOPLASM CELL SURVIVAL

Clifton, K. H., Mahler, P., Watanabe, H., University of Wisconsin, Madison Campus, Center for the Health Sciences, 500 Lincoln Dr., Madison, Wisconsin, 53706, United States of America

The continuing goal of this project is to gather quantitative information on the number, nature, and acute radiation response of the cells involved in initiation of the neoplastic process which leads to mammary tumors following exposure of mammary cells to ionizing radiation in vitro, and how this process is related to the type of radiation, the dose, and the hormonal exposure of the cells before and after irradiation. The project is divided into short-term studies devoted to development and application of a radiation dose-mammary cell survival assay, and to long-term carcinogenesis experiments. The former will be based on grafting of monodispersed mammary cell suspensions into the mammary tissue-free fat pads of rats which are primed with injected hormones and/or grafted with mammotropin-secreting pituitary tumors. Long-term studies will involve irradiation of mammary cell suspensions in vitro, which will then be grafted in similar animals and observed for tumor development. By these methods we hope to: a) Determine the total number of cells of the type from which the malignancy is derived and which survive exposure, b) minimize the abscopal effects of irradiation, and c) develop a system in which both physical and hormonal factors can be manipulated, before and after exposure, for study of cell survival, repair and neoplasia in a quantitative fashion.

310. ENZYMOLOGY OF RADIATION-INDUCED INTESTINAL CARCINOMA

Osborne, J. W., University of Iowa, School of Medicine, Dept. of Radiology, Jessup Hall, Iowa City, Iowa, 52240, United States of America

Animals will be irradiated throughout the coming year to maintain a pool of intestinal tumor-bearing rats. During this period, we will also continue to induce intestinal tumors in other rats with DMH in order to have animals for comparison.

Our preliminary in vitro investigations of the effect of the cyclic nucleotides and prostaglandins upon the growth of the human colon cells will be studied more extensively. Correlations of nucleotide levels with the in vitro synthesis of carcinoembryonic antigen by these cells were recently initiated and will be completed this final year.

During this past year, we reported that hydrolytic activity was elevated in intestinal tumors, thus possibly accounting for the lower cAMP levels. This coming year, we intend to determine the adenylate cyclase activities in the malignancies thus finding whether there is a defect in the synthetic activities of cAMP as well. These results will then be related to similar investigations of the chemically induced tumors where possible. Assays for the prostaglandins in these tissues will also be initiated.

The significance of our preliminary findings of lower intracellular cAMP concentrations in the tumor-sensitized lymphoid cells will be investigated in greater detail. Correlations will be made with the levels of the cyclic nucleotides and the degree of cytotoxicity. We will attempt to determine if there is any relationship of the prostaglandin concentrations in the sensitized

lymphoid cells to the degree of killing measured by the cytotoxicity assay.

BIBLIOGRAPHIC REFERENCES: Stevens, R.H., Brooks, G.P., Osborne, J.W., Englund, C.W., and White, D.W., Lymphocyte cytotoxicity in X-irradiation induced adenocarcinoma of the rat small bowel. I. Measurement of target cell destruction by release of radioiodinated membrane proteins. *J. Natl. Cancer Inst.*, 59:1315-1319, 1977. Lawson, A.J., Wall, D.D., Osborne, J.W., and Stevens, R.H., Adenosine 3',5'-cyclic monophosphate phosphodiesterase activities in the X-irradiation induced rat small bowel adenocarcinoma. *Biochem. Biophys. Res. Comm.*, 78:992-997, 1977.

311. CELL TYPE AT RISK IN CEREBRAL CARCINOGENESIS

Hopewell, J. W., University of Oxford, Research Inst., c/o Churchill Hospital, Oxford, England, United Kingdom

OBJECTIVES: To determine the cells at risk in cerebral carcinogenesis and to examine factors influencing primary glial tumor induction.

APPROACH: Cerebral glial tumors are rare in rats. A high incidence can, however, be induced by the implantation of the carcinogen benzpyrene into the brain. Using this technique a higher incidence of brain tumors is produced in males than in females, the sex ratio being similar to that naturally occurring in man (Hopewell, Edwards and Wiernik, 1976, *Brit. J. Cancer*, 34, 666-670). The effects of cerebral irradiation on glioma induction are now being investigated.

PROGRESS: Histological studies have suggested that tumors of the central nervous system are associated with the subependymal plate (Hopewell, 1975, *J. Path.*, 117, 101-103). Cells in this region, primitive neuroglial precursors, may be the population at risk. Sex hormone levels influence the tumor incidence as a consequence of carcinogenic implantation (Hopewell, 1975, *J. Neuropath. Appl. Neurobiol.*, 1, 141-146), an observation that may be linked to effects on the proliferation characteristics of subependymal cells (Hopewell, 1971, *Cell Tiss. Kinet.*, 4, 273-278).

312. STUDIES ON THE USE OF CARBORANYLALANINE FOR NEUTRON ACTIVATION THERAPY

Schwyzer, R., Eberle, A. N., Kriwaczek, V. M., Eidgenossische Technische Hochschule Zurich, Molekularbiol. Biophysik Inst., Honggerberg, CH8093 Zurich, Switzerland

OBJECTIVE: Use of hormones as specific vehicles for ^{10}B -10 into neoplastic tissues of hormone target organs.

APPROACH: Synthesis of affinity labels of the type: Hormone-(B)-Reactive Group, wherein the hormone is a biologically active hormone analog containing carboranylalanine (B) and a reactive group enabling the compound to be covalently attached to the hormone target cells. Carboranylalanine is B $10\text{C}_2\text{H}_{11}\text{-CH}_2\text{-CH(NH}_2\text{)COOD}$. Decomposition into 2-HE-4, 3-LI-7 and gamma is achieved by irradiation with slow neutrons (E equals 0.25 eV).

BIBLIOGRAPHIC REFERENCES: A. Eberle, O. Leukart, P. Schiller, J.-L. Fauchere, R. Schwyzer, *FEBS Lett.* 82, 325 (1977). A. Eberle, W. Hubscher, R. Schwyzer, *ibid.* 60, 2895 (1977).

313. MODIFYING RADIONUCLIDE EFFECTS

Mahlum, D. D., U.S. Dept. of Energy, Battelle Pacific Northwest Lab., P.O. Box 999, Richland, Washington, 99352, United States of America

The summary for this project is under entry no. 189.

314. NORMAL TISSUE RADIOBIOLOGY - SENSITIVITY OF HORMONE RESPONSIVE CELLS (MICE)

Gould, M. N., University of Wisconsin, Madison Campus, Medical School, Dept. of Human Oncology, 450 N. Randall Ave., Madison, Wisconsin, 53706, United States of America

This is part of a broader project. A summary of this subproject is not available.

3. ROLE OF THE IMMUNE SYSTEM

315. THE CELLULAR AND MOLECULAR BASIS OF ULTRAVIOLET LIGHT IRRADIATION INDUCED EFFECTS ON THE IMMUNE RESPONSE

Greene, M. I., Harvard University, Boston Campus, School of Medicine, Dept. of Pathology, 25 Shattuck St., Boston, Massachusetts, 02115, United States of America

The objective of this study is to examine the effects of ultraviolet (UV) light irradiation on defined elements of the immune system at the cellular and cell membrane level. The work will focus on the observation generated in our laboratory that UV effects are primarily associated with defective antigen presentation cell (APC) function. The methods of procedure are initially to isolate APC from the skin, the spleen and the circulation from normal or UV treated recipients and to characterize the nature and number of I-A ion cells (a marker of APC) using monoclonal and anti-I-Ak antibody probes and the fluorescence activated cell sorter. The second phase of study will analyze the ability to generate suppressor T cells (Ts) to the highly immunogenic polyoma tumor, by immunizing UV treated syngeneic mice with polyoma lysates or virus or viral mutants, and assaying the Ts so generated in vivo and in vitro. The third phase of study involves the use of UV irradiated skin transplants transferred to UV irradiated recipients with the intent of generating Ts to histocompatibility antigens and hence prolonging graft survival.

316. ULTRAVIOLET CARCINOGENESIS AND IMMUNITY

Fortner, G. W., Kansas State University, School of Arts & Sciences, Dept. of Biology, Anderson Hall, Manhattan, Kansas, 66502, United States of America

Epidemiological studies have indicated that some human skin tumors may be a consequence of exposure to the ultraviolet wavelengths of sunlight. Likewise, chronic ultraviolet irradiation induces highly antigenic spindle cell skin tumors on the back of inbred mice. This tumor system offers a unique model for the study of the lymphocyte mediated suppressive effects of ultraviolet light (UVL) as well as anti-tumor immunity in these hosts. The majority of tumors induced by UVL are immunogenic and, thus, rapidly rejected when transplanted into syngeneic hosts; however, UVL-induced tumors grow progressively in autochthonous and syngeneic UVL-irradiated hosts. Suppressor T cell activity and not panimmunosuppression has been established as the mechanism for this tumor progression. Through the use of established in vivo and in vitro methodology studies will be undertaken to determine the basic immune response to UV light-induced murine skin tumors. The primary objective is to gain a better understanding of how tumors regress or progress by studying the tumors in situ. The infiltrating host cells will be analyzed as to type by identification of cell surface markers and as to function by measuring cytotoxicity to tumor targets. A comparison will be made in regressor and progressor mice of both normal and UVL-irradiated origin. The interactions of infiltrating host cells with each other and within the local tumor environment will be analyzed. The relationship between peripheral anti-tumor reactivity and the immunologic response in situ will be determined.

BIBLIOGRAPHIC REFERENCES: Lill, P. H., and Fortner, G. W., Identification and cytotoxic reactivity of inflammatory cells recovered from progressing or regressing syngeneic UV-induced murine tumors, *J. Immunology* 121:1854, 1978.

317. ULTRAVIOLET LIGHT RADIATION AND IMMUNOREGULATION

Daynes, R. A., Utah Higher Education System, University of Utah, School of Medicine, Dept. of Pathology, 1400 E. 2nd St., Salt Lake City, Utah, 84112, United States of America

During the third year of our project we hope to extend our knowledge of syngeneic host-tumor interactions. The UV-tumor model has provided us with an easy way to manipulate a syngeneic system so that either an effector or suppressor mechanism will dominate following tumor challenge. This provides a potential means of investigating numerous questions about immunoregulatory influences and effector cell responses. We propose to study the nature of the effector T-cell-suppressor T-cell interaction and to acquire knowledge of the lymphocyte and

macrophage subpopulations necessary to generate the antitumor immune state. Tumors will be evaluated with respect to the various types of membrane-bound antigens they possess (both TSTA and TAA). The immunologic responses (effector and regulatory) will be studied as they relate to these tumor antigens.

BIBLIOGRAPHIC REFERENCES: Spellman, C.W. and Daynes, R.A. Ultraviolet Light Induced Murine Suppressor Lymphocytes Dictate Specificity of Anti-Ultraviolet Tumor Immune Responses. *Cellular Immunol.* 38:25 (1978). Spellman, C.W. and Daynes, R.A. Immunoregulation by Ultraviolet Light-III. Enhancement of Suppressor Cell Activity in Older Animals. *Exp. Geront.* 13: 141 (1978).

318. CELL-MEDIATED IMMUNOLOGICAL DEFECTS AFTER IRRADIATION

Goh, K., Reddy, M. M., Hempelmann, L. H., University of Rochester, School of Medicine & Dentistry, Dept. of Medicine, 601 Elmwood Ave., Rochester, New York, 14627, United States of America

There is a higher risk of developing cancer in persons who have been exposed to radiation. The mechanisms by which radiation causes cancer are not known. It has been shown that T-lymphocytes are primarily responsible for cell-mediated immunity of the host. These cells are probably also the most important cells in the body as far as the elimination of neoplasms is concerned. Therefore, it is possible that radiation injures the T cells. The aim of the study is to determine subpopulation of peripheral blood lymphocytes in adults whose thymus glands were irradiated prenatally, neonatally, in early infancy and during adult life. The persons to be examined will be recruited from (1) those whose mother had pelvimetry x-ray before birth, (2) those who were irradiated during infancy or early childhood because of enlarged thymus glands, (3) those who have been irradiated for cancer of lung or breast and (4) those who have been treated with radiation for mastitis. It has been estimated that there will be 40 persons from each group, and normal siblings, spouses or hospital staff will be used as controls. Any immunologically related diseases will also be examined.

319. STEM CELL AGE AND X-RAY/CHEMOTHERAPY LEUKEMOGENESIS

Greenberger, J. S., Sidney Farber Cancer Center Inc., 44 Binney St., Boston, Massachusetts, 02115, United States of America

Accumulated age and proliferative stress of the pluripotent hemopoietic stem cell compartment will be studied for its effect on x-ray/chemotherapeutic alkylating agent-induced acute granulocytic leukemia in vitro. RFL mice, a strain demonstrating a high incidence of acute myelogenous leukemia following exposure to total body irradiation and inbred Swiss mice (SIM), a strain with a very low incidence of leukemia will be studied. Serial transfer of bone marrow from normal mice to lethally irradiated recipients will be carried out to stress normal hemopoietic stem cells. Each generation and the initial group of mice will be exposed to a leukemogenic dose of total body irradiation or nitrogen mustard. Bone marrow from treated animals, 3 months after exposure to the cytotoxic agent, will be explanted to an in vitro culture system which has been demonstrated to generate continuous tissue culture lines of acute promyelocytic leukemia. Clonally derived APML cell lines will be characterized for the rapidity with which they are induced in marrow cultures. These studies should determine the effects of: genetic susceptibility to leukemia, accumulated proliferative stress on the stem cell compartment, and recent or delayed exposure to total body irradiation or alkylating agent cytotoxic therapy on the induction of acute myelogenous leukemia. We have demonstrated no spontaneous leukemia in over 400 SIM bone marrow cultures over the last 3 years. None of 36 cultures of RFL c/Na or RFL s/Na have spontaneously generated acute myelogenous leukemia. Studies of the effects of total body irradiation or nitrogen mustard on the first generation of bone marrow donors are in progress.

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7:(suppl. 5), 279-289, 1979. Greenberger, J.S., Sakakeeny, M.A., and Parker, L.M.: In Vitro Proliferation of Hematopoietic Stem Cells in Long-term Marrow Cultures: Principals in Mouse Applied to Man, *Exp. Hem.* 7:(suppl.5), 135-148, 1979.

320. IMMUNE IMPAIRMENT FOR INHALED RADIONUCLIDES

Bice, D. E., Lovelace Center for the Health Sciences, Inhalation Toxicology Research Institute, 5200 Gibson Blvd. S.E., Albuquerque, New Mexico, 87108, United States of America

Chronic pulmonary irradiation from inhaled radionuclides has been shown to severely depress systemic immune responses. This project investigates the effects of irradiation on the function of lymphoid tissues associated with the lung. Inhaled insoluble radioactive particulates tend to accumulate in tracheobronchial lymph nodes and in bronchus-associated lymphoid aggregates. Our studies will determine if an accumulation of radioactive particles in these tissues will result in a loss of local immunologic function even when levels present are not sufficient to produce a systemic depression of immunity. These lymphoid tissues are the first site at which protective immune responses would be produced against antigens released from primary lung tumors. A loss of their function could allow early tumor growth in the lung without recognition by immunosurveillance mechanisms. Current studies will provide data concerning immunologic responsiveness of lymphocytes in lung-associated lymphoid tissues after inhalation exposure to insoluble $^{239}\text{PuO}_2$ particles. At various intervals after exposure to radioactive particles, animals will be immunized by aerosols of particulate antigens and immunologic responses evaluated. Future studies will be designed to determine if animals with compromised lung immunologic mechanisms have a higher incidence of lung tumors following exposure to carcinogens.

321. ELECTRON MICROSCOPE STUDIES ON IRRADIATION OF BLOOD LYMPHOCYTES IN VITRO

Stefani, S. S., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, 5th Ave. & Roosevelt Rd., Hines, Illinois, 60141, United States of America

The ultrastructural changes in human peripheral blood lymphocytes and in cultured bronchogenic carcinoma cells following a lethal hyperthermic exposure (45 degrees centigrade for 1/2 to 1 hour) were compared with those following a lethal simple dose of 4000R of x-rays. It was shown earlier (*Int. J. Radiat. Biol.* 31:215, 1977) that following irradiation a sequence of morphological events took place in the cytoplasm and the nuclei of irradiated cells before the cells disintegrated at about 24 hours. These events were described as a localized protrusion of the plasma membrane, fenestration of the plasma membrane, rarefaction of the cytoplasm, a localized protrusion of the outer nuclear envelope and formation of intranuclear vacuoles. Lymphocytes treated with hyperthermia, on the other hand, exhibited only one change, a condensation of the cytoplasmic and nuclear matrices. Such cells appeared electron opaque in the electron microscope and disintegrated at about 2 hours following the hyperthermic treatment. The bronchogenic carcinoma cells exhibited two modes of cells, one through condensation of the cytoplasm and the nucleus and the other in which there was an increase in the cell size, many large blebs formed on the cell surface and the cytoplasm and the nucleus appeared rarefied. These blebs represented an extension of the cytoplasm. These blebs were different from the protrusion of the plasma membrane observed following a lethal dose of x-rays in which the protruded space contained no cytoplasmic matter. Studies determining the sequence of ultrastructural events following hyperthermic treatment are under way.

322. IMMUNE SURVEILLANCE IN RADIATION CARCINOGENESIS

Anderson, R. E., Troup, G. M., Warner, N. L., Dram, L. S., University of New Mexico, School of Medicine, Dept. of Pathology, University Hill N.E., Albuquerque, New Mexico, 87131, United States of America

Neonatal thymectomy, which inhibits maturation of select aspects of the immune response, renders animals more susceptible to the carcinogenic action of oncogenic viruses and select chemical carcinogens. In man, several thymus-related immunologic deficiency diseases have been shown to be characterized

by a markedly increased frequency of malignant tumors, as has been chronic immunosuppressive therapy.

On the basis of the above and related observations, the recirculating thymic-dependent small lymphocyte (T cell) has been implicated in the pathogenesis of a variety of spontaneous and artificially induced tumors and it has been postulated that this cell type constitutes an integral part of the immune surveillance mechanism. The remarkable radiosensitivity of the small lymphocyte has been well documented, as has been the carcinogenic effect of ionizing radiation. The mechanisms involved in such tumorigenesis are not clear, however, and in particular the relationship between the immunosuppressive effects of exposure to biologically significant amounts of whole body radiation and the neoplastic consequences of such exposure remain poorly defined.

The purpose of the proposal is threefold: 1) to investigate the effect of neonatal and adult thymectomy on the prevalence of spontaneous and radiation-related tumors in germfree and conventional mice; 2) to determine if the tumorigenic effects of radiation can be modified via reconstitution with defined populations of lymphocytes post-exposure; suppressor and helper T cells are of particular interest in this regard; 3) to correlate the above with the immune status of the host, particularly with respect to the number of recirculating T cells mobilizable via thoracic duct cannulation.

Other methods of evaluation include: temporal evaluation of the immunologic capabilities of thymic-dependent lymphocytes from each experimental group including evaluation of relative numbers of suppressor and helper cells; morphologic evaluation of all deaths to include histologic evaluation of tumors; age-specific death rate for neoplasms.

323. REINTERPRETATION OF RADIATION DOSE LYMPHOCYTE SURVIVAL CURVES TAKING ACCOUNT OF CELL DEATH RATES

Vaughan Smith, S. Thomson, A. E., Imperial Cancer Research Fund, Cytochemistry Laboratory, Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom

We have been examining the susceptibility in vitro of non-dividing, diploid normal peripheral blood lymphocyte populations (NHL) to being killed by X rays. Nuclear pyknosis has been used as the indicator (previously validated) of cell death. The sought after numerical measure of radio-sensitivity of a uniformly radiosensitive population or subpopulation is the radiation dose 'R' allowing 37 percent of the cells to survive.

The graphical relationships between the logarithm of the size of the surviving cell fraction and radiation dose (radiation responses) could be drawn essentially linearly biphasic. From experiments with NHL depleted in T cells the less radiosensitive phase could be crudely identified with the T cells and the more radiosensitive phase with the coexisting B cells.

The enumeration of surviving cells as a function of increasing dose had to be done on cells maintained in culture at times after irradiation when survival in control unirradiated cultures remained high. At these times, lethally affected cells were present which had not yet died (turned pyknotic) and being morphologically indistinguishable from true survivors these apparent survivors became included in the dead cell count to an extent governed by their death rate.

Both lethally hit T cells and B cells appear to die by first order kinetics and independently of dose. The half lives for the two cell types varied in the same range as one another (11-63 hr) amongst the donors but independently of one another. These variations accounted considerably for differences in shape of radiation response between donors which could be misconstrued as radiosensitivity differences alone be perfunctory inspection. Calculations made to allow for death rate based on the equation formulated previously (ICRF Scientific Report 1976 - report 113) have given estimates of true radiosensitivity in the range 25-80 R for B cells and in the range 300-400 R for T cells.

324. MICROWAVES, HYPERTHERMIA, AND HUMAN LEUKOCYTE FUNCTION

Roberts, N. J., Michaelson, S. M., Manzella, J. P., Lu, S., University of Rochester, School of Medicine & Dentistry, Dept. of Medicine, 601 Elmwood Ave., Rochester, New York, 14627, United States of America

Microwave radiation has many current uses that result in public exposure, with very little information available on the effects of microwaves on man. Investigators have reported increased spontaneous transformation of lymphocytes from animals that have been exposed to microwaves. These effects are still of unknown functional significance. Quantitation of thermal variables is difficult and critical to understand these effects. The studies of this proposal will determine whether microwave exposure to human mononuclear leukocytes in vitro will similarly affect these cells, as well as determine whether such effects are beneficial or detrimental and whether they are due to microwaves or are thermal in nature. In addition, the studies will explore the effects of microwave-induced hyperthermia on the function of human leukocytes in defense against cancer tissue, since microwaves are being investigated in therapy of cancer patients.

Purified human mononuclear leukocytes, macrophages and lymphocytes will be exposed in vitro to microwaves, singly and in combination, to attain physiologic temperatures (37-40 degrees C) and those used in cancer therapy (40-42 degrees C). The function of these cells, measured by transformation response to mitogen or antigen, will be assayed and compared to that of cells treated concurrently in a like fashion with the exception of microwave exposure. The latter cells would have their temperature set by exposure to appropriate ambient temperature conditions. By recombinations of macrophages and lymphocytes exposed to different temperatures (with and without microwave exposure), followed by testing of response to mitogen stimulation, the cells affected by microwaves and/or hyperthermia can be determined, as well as the significance of such exposure for cell function. Similar exposure of human macrophages and target tumor cells in vitro, with subsequent combination of these cells in the presence and absence of interferon, will determine the effects of microwaves and/or hyperthermia on macrophage function to produce tumor cell cytostasis, determined by measurement of thymidine incorporation by the target cells after treatment.

325. SOMATIC EFFECTS OF PROTRACTED LOW DOSE RADIATION

Goldman, M., University of California, Davis Campus, School of Medicine, Dept. of Medicine, Davis, California, 95616, United States of America

The efficient manner in which chronic gamma-irradiation induces myeloproliferative disorders in beagles is being utilized to investigate mechanisms involved in radiation-induced leukemogenesis. Using a variety of bone marrow, peripheral blood and lymphoid organ cell culture systems developed for the investigation of hematopoiesis and cell mediated immunity in beagles, the 60-Co facility will be utilized to study the following: (1) disturbances in hematolymphopoiesis and promotional aspects of leukemogenesis as reflected in bone marrow culture systems supporting hematolymphopoietic progenitor cells as well as poietin producing populations; (2) the role of candidate mesenchymal progenitors in radiation-induced leukemia; (3) the ability of bone marrow culture techniques to serve as sensitive biological indicators of radiation-induced injury; (4) development of immunological methods for detecting radiation-induced damage; (5) correlation of general and leukemia-specific immune responses to the pathogenesis of radiation-induced disease; and (6) efficacy of bone marrow transplantation and immunotherapy in the radiation-induced leukemia model.

326. INFLUENCE OF GENETIC IMMUNE DISORDERS

Wilson, F. D., Shifrine, M., University of California, Davis Campus, Radiobiology Lab., Davis, California, 95616, United States of America

The basic objective is to determine if the genetic disorders producing immunological or hematological deficiencies enhance susceptibility to radiation-induced leukemia. OSD will use the results in determining the health effects of exposure to low-level radiation and the necessity for establishing standards for susceptible sub-populations. Normal mice and mice with either genetically based immunological disorders or genetic anemias will be exposed to continuous whole body gamma irradiation at 20 r/day. Unirradiated controls will also be maintained. Exposure will continue until leukemia or other radiation-induced diseases are manifested. Animals will be sacrificed for pathology exami-

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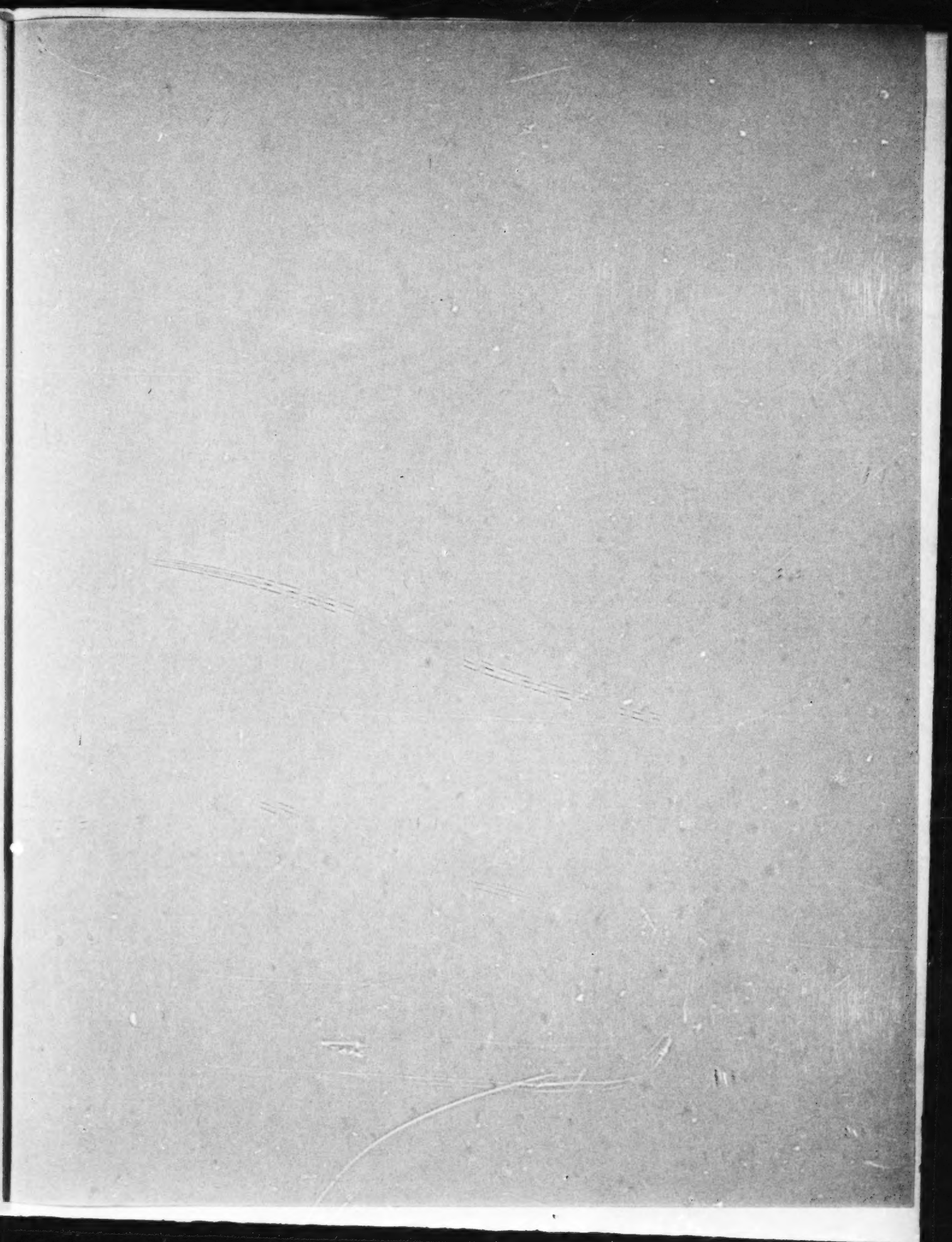
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